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## ERRATA AND AUTHORS' EMENDATIONS

Page 9, footnote *a* of table 8 should be "Calculated by using the efficiency of the basal ration."
Page 48, table 7, heading of first column, "Group" should be "Emulsion No." and heading of second column, "Emulsion No." should be "Sample No."
Page 340, legend for figure 3, "X 120" should be "X 85."
Page 378, table 2, last column, "58.7" should be "58.5."
Page 409, table 3, first column, under Grimes Golden, "New York, 1919" should be "New York, 1929."
Page 519, table 1, eleventh column, average head widths of fourth instar, males, "1.99" should be "1.22."
Page 592 and 593, tables 3 and 4, the percentages of beetles surviving in cages 4 and 7 in 1923-24 should be "4.16" and "23.54", respectively.
Page 748, line 18, "woolly-pot" should be "woolly-pod."
Page 788, figure 6, legend A refers to figure 6, B, and B refers to figure 6, A.
Page 842, table 1, column 5, omit "Per cent."
Page 1025, eighteenth line from bottom, "on" should be "of."
Page 1026, table 1, last column, omit "*Per cent*" under "Ca:P ratio."
Page 1094, fifth line from bottom, "Filco" should be "Difco."
Opposite pages 1104 and 1105, plates 1 and 2 should be transposed, the legends remaining as they are.
Page 1104, fifteenth line from bottom, "Pl." should be "fig."

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# PRODUCTION AND HATCHABILITY OF EGGS AS AFFECTED BY DIFFERENT KINDS AND QUANTITIES OF PROTEINS IN THE DIET OF LAYING HENS[1]

By THEODORE C. BYERLY, *Physiologist;* HARRY W. TITUS, *Biological Chemist;* and N. R. ELLIS, *Chemist, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*

## INTRODUCTION

It is generally accepted that the cereal feedstuffs usually fed to laying hens must be supplemented by some protein concentrate if high egg yields, necessary for the profitable production of eggs and chicks, are to be obtained. Meat, milk, and fish products are commonly used; vegetable-protein concentrates are usually cheaper, but, in general, they are not considered so efficient as the protein concentrates of animal origin. However, most of the investigations on the effect of diet on egg production and hatchability have been purely qualitative. The results of some of them are contradictory.

The experimental work discussed in this paper was carried out at the United States Animal Husbandry Experiment Farm, Beltsville, Md. The purpose of the study was to determine the effect on egg production and hatchability produced by the addition of different protein concentrates to the diet of laying hens. Protein concentrates of both animal and vegetable origin were used; the basal diet with which they were fed contained only feedstuffs of plant origin, a mineral mixture, and cod-liver oil.

This paper presents quantitative estimates of the efficiency of the total protein content of the diet (the percentage of protein ranging from 11.2 to 23.6) and of the relative efficiency of the protein supplements of different origin. To demonstrate the manner in which the protein level affects egg production, correlations are presented between the protein content of the diets and the several factors involved in egg production, such as egg weight and intensity of laying.

Data on the hatchability of the eggs produced and the mortality of the embryos during incubation are presented, together with an analysis of the causes of death, especially as they are related to the diet. In addition, the relationship between current egg production and hatchability is discussed in the light of the data here presented.

## PREVIOUS INVESTIGATIONS

### EFFECT OF DIET ON EGG PRODUCTION

Egg production is correlated with certain hereditary and physiological characters of the hen. Diet may influence egg production indirectly by acting on some character of the hen which affects egg production as well as by direct action on the process of egg formation. The literature on this phase of the subject is summarized in standard

[1] Received for publication Mar. 18, 1932; issued February, 1933.

textbooks, such as those by Lippincott (*17*) [2] and Jull (*13*), and is considered only briefly here.

The age of the pullet at the time she begins to lay has been shown by Maw and Maw to be positively correlated with egg weight (*20*).

The percentage of protein in the diet and the age of the pullet when the first egg is laid are generally supposed to be correlated. Norris and Heuser (*22*) have made a careful study of the growth of chicks on diets containing different percentages of meat scrap. They obtained increased growth per pound of feed eaten as the protein was increased from 13 to 20 per cent but found that the growth per pound of protein decreased steadily. Birds reared on a low-protein diet tend to overtake those reared on a high-protein diet after about 8 weeks of age. Atwood and Clark (*2*) have shown that the age of the pullet when she lays her first egg is negatively correlated with first-year production.

Live weight is positively correlated with egg size, according to Atwood and Clark (*2*) and Hauschildt (*8*). Lamon and Lee (*15*) found that hens on an all-cereal diet and those on diets containing cottonseed meal weighed less than hens on a diet containing meat scrap. The percentage of protein in the diet and live weight probably affect both albumen weight and yolk weight, since it has been found that both albumen weight and yolk weight are closely correlated with egg weight. Live weight and egg production were reported to be positively correlated by the Canadian National Poultry Record Association (*5*). Atwood and Clark (*2*) failed to find a significant correlation between these factors.

Annual production and annual mean egg weight were not found by Atwood and Clark (*2*) to be significantly correlated. On the other hand, Jull (*12*), in an analysis of data from English laying trials, found a distinct tendency for the high producers to lay small eggs. Marble (*18*) found that both high and low producers laid smaller eggs than average producers. In view of the common belief that egg size is inherited, it may very well be that the presence or absence of correlation depends on whether or not the stock studied by the workers cited had been selected for egg size.

The percentage of protein in the diet and the intensity of egg production are correlated, according to the work of Buss (*3*), Philips (*26*), Kempster (*14*), and Martin (*19*). Kempster (*14*) obtained a correlation coefficient of $+0.5179 \pm 0.071$ between egg production and the percentage of meat scrap in the diet; the diets contained from 5 to 34 per cent of meat scrap in the mash part. He obtained as good production with 25 as with 34 per cent. Parkhurst (*23*) and Walker (*29*) found that feed cost of egg production decreased as the amount of protein in the diet was increased.

There is an extensive literature on the relative efficiency of protein supplements for egg production from the qualitative standpoint. The vegetable-protein concentrates are generally considered to be deficient for egg production. Animal-protein supplements are usually recommended by the agricultural experiment stations. Much work has been done on the feeding of meat products, fish meals, and milk products to determine the best levels and methods of feeding.

The feeding of milk in its various forms is universally recommended for egg production, and many experiments have been made on the methods of feeding and the quantities to be fed.

[2] Reference is made by number (italic) to Literature Cited, p. 21.

### EFFECT OF DIET ON HATCHABILITY OF EGGS

Heuser (*9*) reported that high antecedent winter and high current egg production during the hatching season reduced hatchability unless the hen was on a sufficiently good diet to maintain body weight and vigor. Jull (*11*) was unable to find any significant correlation between hatchability and egg production during the 90 days preceding the hatching season. Atwood and Clark (*2*) found no correlation between egg weight and hatchability.

Atwood (*1*) reported that so far as hatchability is concerned, it is immaterial whether the breeding hens receive a high-protein or a low-protein diet before the breeding season.

Payne (*24*) and Byerly (*4*) have reported considerable data on the time of occurrence of mortality during incubation. Payne (*24*) reported peaks in the mortality curve on the fourth and nineteenth days of the incubation period. Byerly (*4*) found that the early peak in the mortality curve for his material occurred on the third day, and he also reported the frequent occurrence of a peak in the curve at about the middle of the incubation period.

Dunn (*6, 7*) and Landauer (*16*) described a condition of the embryo which typically involves parrotlike beaks and shortened legs with bent tibia. Landauer (*16*) showed that this condition resembles chondrodystrophy, or achondroplasia, in mammals. It was, therefore, called chondrodystrophy. Dunn (*6*) advanced the hypothesis that it was inherited as a simple Mendelian recessive character. His later report (*7*) did not support this view, for it was found that close inbreeding did not increase the incidence of chondrodystrophy. Dunn (*7*) found that chondrodystrophy appeared more frequently early in the breeding season than later and suggested that lack of ultra-violet light might be involved. Hutt and Greenwood (*10*) found a similar distribution of chondrodystrophy in their material and suggested that the condition is induced by lack of sunlight accentuating a hereditary tendency to the defect.

### MATERIAL AND METHODS OF THE EXPERIMENT

The chickens used were Single-Comb Rhode Island Red pullets and cockerels. The pullets were distributed in two series of pens, nine pens in each series. In one series, each pen contained 25 pullets; in the other, each contained 15. The pullets were distributed according to weight, so that, as shown in Table 3, there were no significant differences in the mean weights of the birds on the several diets. On the south side of each pen large apertures covered with wire netting let in the sunlight. The birds had access to a small concrete-floored yard on the south side of each pen. Straw litter was used and the pens were kept scrupulously clean. The birds were trap-nested; four trips were made each day to record the eggs and release the birds from the nests. The cockerel in each pen was transferred from one pen to the next in the series each day.

In compounding the several diets a basal feed mixture was used which consisted of the following:

| | Parts by weight |
|---|---|
| Yellow corn meal | 500 |
| Wheat bran | 245 |
| Rolled oats | 150 |
| Alfalfa-leaf meal | 55 |

The basal diet consisted of the following:

| | Parts by weight |
|---|---|
| Basal feed mixture | 95 |
| Mineral mixture | 5 |
| Cod-liver oil | 2 |

The mineral mixture consisted of the following:

| | Parts by weight |
|---|---|
| Ground limestone | 50 |
| Steamed bone meal | 30 |
| Anhydrous sodium sulphate | 10 |
| Common salt | 10 |

For convenience, the cod-liver oil was mixed with a part of the corn meal before the mixing of the complete diet. The other eight diets consisted of the following:

| | Parts by weight |
|---|---|
| Basal feed mixture | 75 |
| Protein supplement | 20 |
| Mineral mixture | 5 |
| Cod-liver oil | 2 |

The average partial chemical analyses of the protein supplements used in the diet are shown in Table 1.

TABLE 1.—*Average partial chemical analyses (per cent) of the protein supplements used in the diet*

| Diet No. | Protein supplement | Water | Protein [a] | Ash | Fat |
|---|---|---|---|---|---|
| 1 | Desiccated meat meal [b] | 8.14 | 72.44 | 6.75 | 10.33 |
| 2 | Crab scrap meal | 7.84 | 35.06 | 28.84 | 2.50 |
| 3 | North Atlantic fish meal | 10.96 | 69.06 | 19.83 | 1.46 |
| 4 | Dried buttermilk | 8.52 | 33.79 | 7.12 | [c] 1.51 |
| 5 | Dried-yeast preparation [d] | 10.96 | 10.94 | 1.68 | 2.62 |
| 6 | Soybean meal [e] | 8.12 | 47.36 | 5.34 | 6.30 |
| 7 | Cottonseed meal | 9.24 | 33.87 | 5.75 | 6.90 |
| 8 | Desiccated meat meal, 8 parts, by weight; North Atlantic fish meal, 7 parts; dried buttermilk, 5 parts | [f] 9.15 | [f] 61.59 | [f] 11.42 | [f] 5.02 |

[a] N×6.25.
[b] Not available commercially.
[c] Low average fat content indicates that much of the so-called buttermilk used was processed skim milk. Laboratory analyses of the bureau were not available until the experiment was under way.
[d] Yeast dried with corn meal, a commercial product for animal feeding.
[e] The term "soybean meal" refers to the product obtained by grinding the soybean oil cake.
[f] Calculated from values given for components.

The average partial analyses of the diets as finally mixed and fed are given in Table 2. All feed was given as dry mash in "reel" feed hoppers, and the birds had access to it at all times.

TABLE 2.—*Average partial chemical analyses (per cent) of the diets, as fed*

| Diet No. | Water | Protein | Ash | Fat | Fiber |
|---|---|---|---|---|---|
| 1 | 12.62 | 23.56 | 7.15 | 7.66 | 4.00 |
| 2 | 11.25 | 15.64 | 11.28 | 5.33 | 5.80 |
| 3 | 11.63 | 22.75 | 8.99 | 5.71 | 3.65 |
| 4 | 11.47 | 15.35 | 6.38 | 5.84 | 3.57 |
| 5 | 11.41 | 11.21 | 6.23 | 6.57 | 4.13 |
| 6 | 11.14 | 18.34 | 6.63 | 6.59 | 4.62 |
| 7 | 11.16 | 15.86 | 6.54 | 6.71 | 6.59 |
| 8 | 11.45 | 20.62 | 6.56 | 6.27 | 3.52 |
| 9 | 10.84 | 11.53 | 5.77 | 6.10 | 5.00 |

Weekly records of feed consumption and weight of the birds were kept for the 25-bird series of pens. Records were kept for 4-week periods for the 15-bird series. All feed consumption was figured on the basis of hen-days, and an allowance was made for the feed eaten by the cockerels. Each egg was weighed on the day that it was laid. The experiment covered a period of 344 days; it was begun on September 3, 1929, and terminated on August 12, 1930.

## EXPERIMENTAL RESULTS

### EFFECT OF DIET ON AGE AT WHICH FIRST EGG WAS LAID

The pullets used were approximately of the same age. Distribution in the several pens according to weight, so that no significant differences existed between the mean weights of the birds in any two pens (Table 3), probably resulted in there being no significant differences in age between the birds in any two pens.

The difference between the average time before the first egg was produced by birds receiving the basal diet and those receiving each of the other diets is significant only in the case of diet 7, which contained cottonseed meal as a protein supplement. The weights of the birds on diet 7 fell below those of the birds on the other diets at about the time the average bird on the other diets began to lay. The feed consumption of the birds on diet 7 was lower than that of the birds on any of the other diets at this period. Lamon and Lee (*15*) found that cottonseed meal apparently lacks palatability. This lack of palatability may account for the significantly greater age at first egg of the birds on diet 7 in the present experiment.

TABLE 3.—*Average number of days before first egg, and average initial, maximum, and final live weights of the birds on each diet*

| Diet No. | Average number of days before first egg | Difference in time before first egg of birds on indicated diet and on diet 9 | Average initial weight of birds | Difference in average initial weight of birds on indicated diet and on diet 9 | Average maximum weight of birds | Difference in average maximum weight of birds on indicated diet and on diet 9 | Average final live weight of birds | Difference in average final weight of birds on indicated diet and on diet 9 |
|---|---|---|---|---|---|---|---|---|
| | *Days* | *Days* | *Grams* | *Grams* | *Grams* | *Grams* | *Grams* | *Grams* |
| 1 | 79.4±3.91 | −14.1±6.0 | 1,770±30.2 | +48±39.0 | 2,691±38.3 | −2±54.9 | 2,437±41.1 | +316±53.0 |
| 2 | 90.3±6.01 | −3.2±7.0 | 1,748±24.8 | +26±35.1 | 2,689±30.7 | −4±49.9 | 2,373±37.6 | +252±50.3 |
| 3 | 96.5±4.90 | +3.0±6.7 | 1,785±25.3 | +63±35.4 | 2,799±31.1 | +106±50.2 | 2,462±36.8 | +341±49.7 |
| 4 | 80.3±4.51 | −13.2±6.0 | 1,771±21.0 | +49±32.5 | 2,757±36.8 | +64±53.9 | 2,479±40.2 | +358±52.3 |
| 5 | 80.5±4.53 | −13.0±6.1 | 1,807±22.7 | +85±33.6 | 2,650±38.3 | −43±54.9 | 2,106±33.4 | −15±47.2 |
| 6 | 83.5±4.31 | −10.0±6.6 | 1,808±26.3 | +86±36.1 | 2,681±28.5 | −12±48.6 | 2,417±38.3 | +296±50.8 |
| 7 | 119.3±6.30 | +25.8±7.3 | 1,761±25.9 | +39±35.9 | 2,618±35.4 | −75±52.9 | 2,202±33.1 | +81±47.0 |
| 8 | 89.4±5.20 | −4.1±7.08 | 1,728±23.3 | +6±34.0 | 2,794±30.2 | +101±49.6 | 2,486±30.1 | +365±45.0 |
| 9 | 93.5±4.81 | | 1,722±24.8 | | 2,693±39.4 | | 2,121±33.4 | |

There is no evidence of correlation between the number of days before first egg and the percentage of protein in the diet. Even the basal diet contained sufficient protein to permit normal assumption of egg laying.

### EFFECT OF DIET ON WEIGHT OF BIRDS

The average maximum weights, shown in Table 3, were observed on February 19, except those of the birds on diet 1, which were at-

tained about a month later. The differences in average approximate maximum weight between the birds on diet 9 and those on the other diets are not significant. The average maximum weights of the birds on diet 5 and on diet 7 were significantly lighter than those of the birds on diet 3. The difference between the average maximum weight of the birds on diet 5 and those on diet 3 was $149 \pm 49.0$ g, and between the average for those on diet 7 and those on diet 3, $181 \pm 47.1$ g. Both of these differences are more than three times their probable errors and therefore are significant.

The birds on diets 1, 2, 3, 4, 6, and 8 had significantly heavier final live weights than those on diet 9. The weights began to diverge when laying commenced and the differences in weight became greater as the experiment progressed. A comparison of the final live weights (Table 3) with percentage of protein in the diets (Table 2) shows a pronounced positive correlation between final live weight and percentage of protein in the diet. The value of this correlation is $r = +0.355 \pm 0.0341$, and being ten times its probable error, it is very significant. This means that pullets laying eggs are able to maintain their weight more readily on diets containing a high percentage of protein, at least within the limits of the percentages used in this experiment. On diet 4 the weight of the birds was more effectively maintained than would have been expected from its protein content.

### EFFECT OF DIET ON WEIGHT OF EGGS

The average egg weights for the hens on diets 1, 3, 4, 6, and 8 are significantly greater than the average egg weight for the hens on diet 9. (Table 4.) A comparison of the values for egg weight with the percentage of protein in each diet shows that they are positively correlated. The coefficient of correlation is $r = +0.339 \pm 0.0317$, which, being more than ten times its probable error, is very significant. Here, too, diet 4 was more efficient than would be expected from its protein content. The eggs from the hens on this diet were heavier than those from the hens on diets 2 and 7, which had the same percentage of protein. They were as heavy as those from the hens on diet 6, which had 3 per cent more protein.

TABLE 4.—*Average weight and total number of eggs produced by the hens on each diet* [a]

| Diet No. | Average egg weight | Difference in average weight of eggs from birds on indicated diet and on diet 9 | Total eggs produced | Average hen-days | Birds alive August, 1930 |
|---|---|---|---|---|---|
| | *Grams* | *Grams* | *Number* | *Number* | *Number* |
| 1 | 57.8±0.378 | +2.9±0.550 | 5,154 | 327.1±4.98 | 34 |
| 2 | 56.1± .394 | +1.2± .561 | 4,917 | 328.4±3.53 | 35 |
| 3 | 59.1± .388 | +4.2± .557 | 4,818 | 320.3±6.44 | 33 |
| 4 | 57.3± .371 | +2.4± .545 | 4,493 | 323.6±4.69 | 30 |
| 5 | 54.9± .350 | ±0 ± .531 | 3,764 | 324.6±5.28 | 33 |
| 6 | 57.0± .367 | +2.1± .542 | 4,837 | 331.6±5.45 | 34 |
| 7 | 56.0± .359 | +1.1± .537 | 3,540 | 325.4+7.57 | 37 |
| 8 | 58.3± .347 | +3.4± .529 | 5,719 | 337.4±3.22 | 38 |
| 9 | 54.9± .399 | ---------- | 4,177 | 335.9±2.07 | 33 |

[a] All averages given are unweighted averages of the average for each hen in each pen.

Egg weight is also positively correlated with body weight. In the present experiment the correlation coefficient for egg weight and body weight is $r = +0.454 \pm 0.0309$.

Since the diet affects the weight of the egg, it affects the weight of the yolk or albumen, or both. The values of yolk weight and albumen weight given in Table 5 are unweighted averages of the averages of one to five eggs for each hen, taken at different times throughout the year, and are therefore only approximately representative. The average yolk weights for the birds on diets 1, 3, and 8 are significantly greater than the average yolk weight for the birds on diet 9. The weights of albumen in the eggs from the birds on diets 1, 3, 4, 6, 7, and 8 are significantly greater than those in the eggs from the birds on diet 9.

TABLE 5.—*Average weight of yolk and albumen of eggs from hens on each diet*

| Diet No. | Average yolk weight | Difference in yolk weight of eggs from birds on indicated diet and on diet 9 | Average albumen weight | Difference in albumen weight of eggs from birds on indicated diet and on diet 9 |
|---|---|---|---|---|
| | *Grams* | *Grams* | *Grams* | *Grams* |
| 1 | 17.09±0.212 | +1.19±0.274 | 32.24±0.401 | +2.33±0.563 |
| 2 | 16.06± .186 | +.16± .254 | 31.07± .359 | +1.16± .534 |
| 3 | 16.81± .198 | +.91± .263 | 32.11± .401 | +2.20± .563 |
| 4 | 16.11± .188 | +.21± .253 | 31.71± .352 | +1.80± .529 |
| 5 | 15.95± .291 | +.05± .339 | 30.25± .339 | + .34± .520 |
| 6 | 16.59± .166 | +.69± .240 | 31.72± .305 | +1.81± .498 |
| 7 | 16.06± .225 | +.16± .284 | 31.75± .458 | +1.84± .605 |
| 8 | 16.68± .160 | +.78± .235 | 32.21± .318 | +2.30± .507 |
| 9 | 15.90± .173 | | 29.91± .395 | |

The coefficient of correlation between yolk weight and egg weight is $r = +0.405 \pm 0.0326$, and between albumen weight and egg weight, $r = +0.759 \pm 0.0165$. There is, of course, a significant correlation between yolk weight and albumen weight, but the coefficient is surprisingly low: $r = +0.312 \pm 0.0220$. The coefficient of correlation between percentage of protein in the diet and albumen weight is $r = +0.148 \pm 0.0384$, and between percentage of protein and yolk weight is $r = +0.212 \pm 0.0374$. These values are probably low because of the small number of each hen's eggs used as a sample.

### EFFECT OF DIET ON INTENSITY OF PRODUCTION

The total number of eggs produced by the hens on each diet is given in Table 4. These figures do not present a true picture of the production of the individual hens, since they are not corrected for mortality and other factors. The number of deaths varied from 2 on diet 8 to 10 on diet 4 (Table 4), but the difference in mean days per hen for these two diets, $13.8 \pm 5.69$, is not significant. The hens were apparently able to survive about equally well on each of the diets. The values for intensity of production, obtained in the manner indicated and shown in Table 4, are too low. The pullets were on the diets for some time before they commenced to lay. The efficiency of the diets for sustaining production is more adequately shown in Table 6. The true values for yearly egg production, however, would

be somewhat lower than those in Table 6, because percentage of egg production usually drops toward the end of the first laying year, which the experiment did not include. The birds on diets 1, 2, 3, and 8, as shown in this table, laid at a significantly greater rate than those on diet 9.

TABLE 6.—*Average annual egg production of hens on each diet* [a]

| Diet No. | Production based on average number of hen-days after first egg | Difference in average production of birds on indicated diet and on diet 9 | Eggs per hen per pullet year (calculated on production after first egg) | Diet No. | Production based on average number of hen-days after first egg | Difference in average production of birds on indicated diet and on diet 9 | Eggs per hen per pullet year (calculated on production after first egg) |
|---|---|---|---|---|---|---|---|
| | *Per cent* | *Per cent* | *Number* | | *Per cent* | *Per cent* | *Number* |
| 1 | 53.1±2.15 | +10.0±2.65 | 197.0 | 6 | 47.3+1.88 | +4.2±2.44 | 173.0 |
| 2 | 51.3±2.08 | +8.2±2.60 | 187.5 | 7 | 43.6±1.71 | +.5±2.31 | 159.5 |
| 3 | 51.6±2.03 | +8.5±2.55 | 188.1 | 8 | 55.9±1.72 | +12.8±2.32 | 204.5 |
| 4 | 46.0±2.30 | +2.9±2.77 | 168.0 | 9 | 43.1±1.55 | | 157.8 |
| 5 | 39.9±1.22 | −3.2±1.97 | 145.8 | | | | |

[a] The averages given in this table are unweighted averages of the average for each hen in each pen.

The data collected in the present experiment show positive correlation, $r = +0.226 \pm 0.0347$, between percentage of protein in the diet and percentage of production after first egg, in spite of the fact that proteins of different origin were used. The correlation is considerably lower than that reported by Kempster (*14*), $r = +0.5179 \pm 0.071$, but he correlated pen averages, whereas the present correlation is for individual hens. In his experiments different percentages of the same protein concentrate, meat meal, were used.

### EFFECT OF DIET ON WINTER EGG PRODUCTION

From the economic standpoint, it is necessary that a diet support winter egg production. The number of eggs produced before March 3 and the percentage which they are of the total eggs produced are given in Table 7.

TABLE 7.—*Winter egg production of hens on each diet*

| Diet No. | Eggs laid from September 4 to March 3 | | Diet No. | Eggs laid from September 4 to March 3 | |
|---|---|---|---|---|---|
| | Number | Per cent of total | | Number | Per cent of total |
| 1 | 2,046 | 39.5 | 6 | 1,759 | 36.5 |
| 2 | 1,466 | 29.8 | 7 | 885 | 25.0 |
| 3 | 1,684 | 35.0 | 8 | 1,919 | 33.4 |
| 4 | 1,948 | 43.4 | 9 | 1,571 | 37.5 |
| 5 | 1,464 | 39.0 | | | |

The birds on diet 7 produced few winter eggs in proportion to the total number produced. The birds on diet 4 laid more eggs during the winter than would be expected from the protein content of the diet, but in the spring and summer this diet proved distinctly less efficient, which confirms the results of Pederick and Clark (*25*).

EGG PRODUCTION PER UNIT WEIGHT OF FEED AND OF PROTEIN

Table 8 gives the egg production per gram of feed and per gram of protein eaten. The supplements used increase in efficiency rather regularly with increase of protein content. The supplement in diet 8 is more efficient than would be expected from the protein content of the three concentrates of which it was composed. This increased efficiency is attributed to the mutual supplementary action of these concentrates.

TABLE 8.—*Egg production of hens, per unit weight of feed and of protein, when on each diet*

| Diet No. | Eggs produced per hen per day after average date of first egg | Feed per hen per day after first egg | Eggs produced per gram of feed eaten | Eggs [a] produced per gram of supplement eaten | Eggs produced per gram of protein eaten | Eggs [a] produced per gram of supplemental protein eaten |
|---|---|---|---|---|---|---|
| | *Grams* | *Grams* | *Gram* | *Gram* | *Grams* | *Gram* |
| 1 | 30.7 | 134.2 | 0.229 | 0.380 | 0.990 | 0.585 |
| 2 | 28.9 | 136.8 | .212 | .263 | 1.348 | .828 |
| 3 | 31.1 | 134.9 | .231 | .357 | 1.012 | .538 |
| 4 | 26.4 | 128.6 | .205 | .235 | 1.282 | .775 |
| 5 | 21.8 | 126.6 | .172 | .073 | 1.540 | .731 |
| 6 | 26.9 | 122.8 | .219 | .305 | 1.198 | .671 |
| 7 | 24.4 | 127.8 | .191 | .167 | 1.190 | .500 |
| 8 | 32.6 | 128.8 | .251 | [b].474 | 1.222 | .831 |
| 9 | 23.6 | 119.2 | .198 | ---------- | 1.720 | ---------- |

[a] Calculated by use of the efficiency basal ration.

[b] Efficiency of supplement in diet 8 calculated from the efficiencies of supplements in diets 1, 3, and 4 is 0.333; the difference, 0.141, is attributed to the supplementary action of the three concentrates on one another.

The efficiency of the protein decreases as the percentage fed increases. The efficiency of the protein in the supplement of diet 8 is somewhat higher than would be expected from the percentage fed. The efficiency of the protein of the supplement in diet 2 is worthy of note. It gave 0.828 g of egg per gram of supplemental protein eaten as compared with 0.775 g per gram of supplemental protein for the supplement in diet 4.

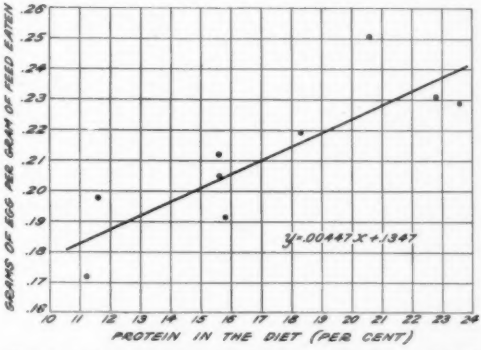


FIGURE 1.—Increase in egg production per gram of feed eaten with increased percentage of protein in the diet. The curve represents the values given by the least square solution of the formula $Y=ax+b$, in which $Y$=grams of egg per gram of feed eaten, $x$=percentage of protein in the diet, and $a$ and $b$=constants. The o's represent the observed values

Egg production per gram of feed eaten plotted against percentage of protein in the diet is shown in Figure 1. Figure 2 shows the egg production per gram of protein eaten plotted against percentage of protein in the diet. It has been pointed out that Norris and Heuser (*22*), in feeding experiments with growing chicks, found a

decreased efficiency of protein with increased protein in the diet. Their hypothesis, that the protein of meat meal, which they used as a supplement, was deficient in essential amino acids, certainly does not explain the decreasing efficiency of protein with increased protein level in egg production.

The data plotted in Figure 2 are taken from 8 experiments carried out in 6 States with 3 breeds of chickens. Twelve protein supplements were used: Meat meal of two kinds, tankage, fish meal, dried buttermilk, sour skim milk, whey, crab scrap, yeast product, cottonseed meal, soybean meal, pea meal, combinations of several of those listed, and several unsupplemented cereal diets. The low values for the basal rations of Lee,[3] Kempster (*14*), and Philips (*27*) are more probably due to the low feed consumption of the birds on these

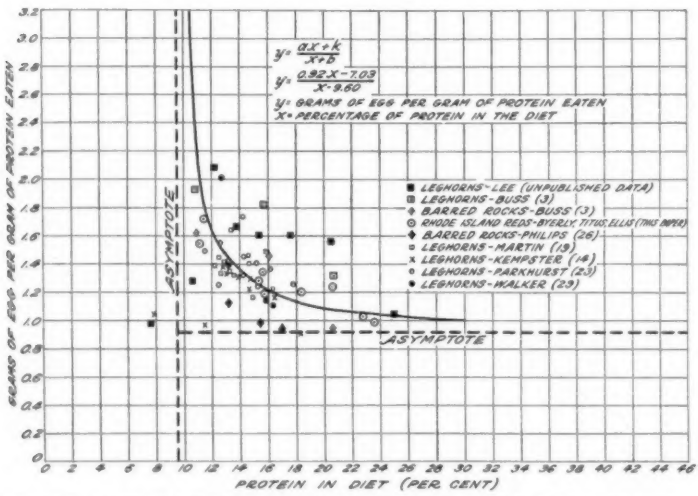


FIGURE 2.—Decrease of protein efficiency with increased protein content in the diet. The formula given describes an equilateral hyperbola and was fitted only to the data in the present experiment by the method of least squares. For further explanation see text

diets than to a relatively low protein efficiency, for in the present experiment the basal ration had the highest protein efficiency of any of the diets used. Feed consumption of birds on this diet was practically the same as that on the other diets, whereas the feed consumption of Lee's birds was very low. Some of the higher values obtained when between 15 and 20 per cent of protein was fed are probably due to stimulated feed consumption rather than to high protein efficiency. In general, the diets containing milk have a somewhat higher protein efficiency for the percentage used than those that do not contain milk. It is remarkable that these data from such widely scattered sources should show so great a degree of similarity.

The curve plotted is fitted only to the data for the present experiment and no rational significance is implied. It seems certain that for diets on which the feed consumption is approximately equal per

[3] LEE, A. R. Unpublished data. 1925.

unit weight of bird, the efficiency of any protein for egg production decreases with increased protein content in the diet, at least between the limits of 11 and 24 per cent. Mitchell (*21*) found that the biological value of proteins decreases with increased percentage of protein in the diet, regardless of protein intake, in the case of milk, corn, and oat proteins fed to rats. Since this is apparently true for proteins generally fed to laying hens, Norris and Heuser's (*22*) hypothesis can not hold. Mitchell disposes of the argument which they advance in the paper cited. The following hypothesis for the decreased efficiency is very similar to Mitchell's. Specific dynamic action may account for part of the decrease. It may be that there is a threshold value for amino acids in relation to concentration of other nutrients in the blood and body fluids above which they are deaminized. If this be true, it would follow that above the threshold value more and more of the amino acids would be destroyed, and relatively less, though in absolute terms more, would pass into the egg. The efficiency need not decrease as the maintenance value is approached, for a hen may lay eggs for a time on a submaintenance ration, as is shown by Titus (*28*).

### CORRELATION BETWEEN EGG-PRODUCTION FACTORS

It has been shown that within the limits of the protein levels used in this experiment, percentage of protein is positively correlated with factors in egg production such as intensity of laying, egg weight, yolk weight, and possibly albumen weight. Significant positive correlation exists between percentage of protein in the diet and body weight; body weight is closely correlated with egg weight.

Table 9 shows that egg weight and percentage of protein in the diet, egg weight and final body weight, egg weight and yolk weight, and egg weight and albumen weight are positively and significantly correlated, as has been pointed out. The correlation between egg weight and intensity of production is positive but not significant in contrast to Jull's (*12*) report of negative correlation between these factors. Final body weight, intensity, yolk weight, and possibly albumen weight, are positively and significantly correlated with percentage of protein in the diet. Yolk weight, albumen weight, and intensity are positively correlated with final body weight. Yolk weight and albumen weight are also positively and significantly correlated.

TABLE 9.—*Zero-order correlations between the factors in egg production affected by protein content of the diet*

| Factor No. | Egg-production factor | Correlation between factors indicated | | | | |
|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 6 |
| 1 | Egg weight | +0.339±0.0317 | +0.454±0.0309 | +0.087±0.0366 | +0.405±0.0326 | +0.759±0.0165 |
| 2 | Percentage of protein | | +.355± .0341 | +.226± .0347 | +.212± .0374 | +.148± .0384 |
| 3 | Final body weight | | | +.149± .0360 | +.274± .0360 | +.298± .0360 |
| 4 | Intensity * | | | | +.053± .0391 | +.002± .0385 |
| 5 | Yolk weight | | | | | +.312± .0220 |
| 6 | Albumen weight | | | | | |

* "Intensity" is used as a brief term denoting percentage of production after the average time the hens on a diet had laid their first eggs.

Zero-order correlations may be only apparent. For example, the correlation between egg weight and protein may be present only because of the effect of percentage of protein on body weight, since egg weight and body weight are closely correlated. To get a more nearly accurate picture, it is necessary to find the net correlations; these are given in Table 10.

TABLE 10.—*Net correlations of factors in egg production*

| Factor No. | Factors in egg production | Net correlation coefficients |
|---|---|---|
| $r_{12.34}$ | Egg weight and percentage of protein, with body weight and intensity held constant. | +0.219±0.0357 |
| $r_{13.24}$ | Egg weight and body weight, with percentage of protein and intensity held constant. | +.380± .0321 |
| $r_{14.23}$ | Egg weight and intensity, with percentage of protein and body weight held constant. | −.019± .0375 |
| $r_{15.234}$ | Egg weight and yolk weight, with percentage of protein, body weight, and intensity held constant. | +.361± .0327 |
| $r_{16.2345}$ | Egg weight and albumen weight, with percentage of protein, body weight, intensity, and yolk weight held constant. | +.736± .0171 |
| $r_{23.14}$ | Percentage of protein and body weight, with egg weight and intensity held constant. | +.222± .0357 |
| $r_{24.13}$ | Percentage of protein and intensity, with egg weight and body weight held constant. | +.187± .0363 |
| $r_{25.34}$ | Percentage of protein and yolk weight, with body weight and intensity held constant. | +.128± .0369 |
| $r_{26.345}$ | Percentage of protein and albumen weight, with body weight, intensity, and yolk weight held constant. | −.006+ .0375 |
| $r_{34.12}$ | Live weight and intensity, with egg weight and percentage of protein held constant. | +.078± .0372 |
| $r_{35.24}$ | Body weight and yolk weight, with percentage of protein and intensity held constant. | +.218± .0357 |
| $r_{36.245}$ | Body weight and albumen weight, with percentage of protein, intensity, and yolk weight held constant. | +.232± .0354 |
| $r_{45.23}$ | Intensity and yolk weight, with percentage of protein and body weight held constant. | −.011± .0375 |
| $r_{46.235}$ | Intensity and albumen weight, with percentage of protein, body weight, and intensity held constant. | −.054± .0375 |
| $r_{56.234}$ | Yolk weight and albumen weight, with percentage of protein, body weight, and intensity held constant. | +.244± .0354 |

The net correlations show that egg weight, final body weight, and intensity are positively and significantly correlated with percentage of protein in the diet. There is a slight correlation between yolk weight and percentage of protein but none between albumen weight and percentage of protein. Intensity of production is correlated significantly only with percentage of protein in the diet. The correlation coefficients between intensity and egg weight, yolk weight, and albumen weight are all negative, but none of them is significant. This stock has been selected for several generations for both egg weight and intensity.

The primary purpose here is to find how much one may expect to increase the production of eggs, thereby decreasing the feed cost of egg production, by increasing the protein content of the diet within the limits of the percentages used in the experiment. Percentage of protein is an independent variable and egg weight, body weight, and intensity are the dependent variables with respect to percentage of protein. The net correlations for these factors may be interpreted in terms of changed egg production or feed cost of egg production by using the regression equation of the dependent on the independent variables in each case and summing the results. The regression of egg weight on body weight, intensity of production on body weight, and egg weight on intensity of production must also be used.

The type equation is

$$Y-\overline{Y}=r\frac{\sigma Y1.234}{\sigma X2.134}(X-\overline{X}),$$

in which $Y$ is the value of the dependent variable corresponding to a selected value of the independent variable $X$, $\overline{Y}$ is the mean value for the dependent variable, $r$ is the net correlation coefficient, $\sigma Y$ is the standard deviation about $\overline{Y}$, $\sigma X$ is the standard deviation about $\overline{X}$, when egg weight, intensity, and body weight are constant. $\overline{X}$ is the mean for the values of the independent variable and $X$ the selected value for the independent variable.

If the lowest and highest observed or derived values for the independent variables are used, the demonstration may be made by the following use of the regression equations.

For egg weight ($Y$) on percentage of protein ($X$):
$Y=0.187X+53.66.$
When $X=11.2$ per cent, $Y=55.75$ g, and when $X=23.6$ per cent, $Y=58.07$ g, the increment being $+2.32$ g---------- (*a*)
For body weight ($Y$) on percentage of protein ($X$):
$Y=17.8X+20.3.$
When $X=11.2$ per cent, $Y=2,219$ g, and when $X=23.6$ per cent, $Y=2,440$ g, the increment being $+221$ g---------- (*b*)
For egg weight ($Y$) on the derived body weight ($X$):
$Y=0.00407X+46.38.$
When $X=2,229$ g, $Y=55.45$ g, and when $X=2,450$ g, $Y=56.35$ g, the increment being $+0.90$ g---------- (*c*)
For intensity of production ($Y$) on percentage of protein ($X$):
$Y=0.825X+33.91.$
When $X=11.2$ per cent, $Y=43.15$ per cent, and when $X=23.6$ per cent, $Y=53.38$ per cent, the increment being $+10.23$ per cent---------- (*d*)

The coefficients of correlation between intensity and egg weight and between body weight and intensity are very small, and since they are not significant might be ignored, but for the sake of completeness they are given.

For intensity of production ($Y$) on body weight ($X$):
$Y=0.00431X+38.09.$
When $X=2,229$ g, $Y=47.70$ per cent, and when $X=2,450$ g, $Y=48.65$ per cent, the increment being $+0.95$ per cent---- (*e*)
For egg weight ($Y$) on intensity of production ($X$):
$Y=-0.0037X+57.05.$
When $X=42.25$ per cent, $Y=56.89$ g, and when $X=53.38$ per cent, $Y=56.85$ g, the increment being $-0.04$ g---------- (*f*)

Thus, increasing the protein content from 11.2 to 23.6 per cent would be expected to increase the annual mean egg weight by 2.32 g (*a*) by direct effect and by 0.90 g (*c*) indirectly by increasing body weight. The increase in protein would increase the intensity of production by 10.23 per cent (*d*), and the increase in body weight would increase the intensity by 0.95 per cent (*e*), making a net increase of 11.18 per cent as compared with the observed increase of 13.2 per cent between diet 5 and diet 1. (Table 6.) The increase in intensity would tend to decrease weight by 0.04 g (*f*), leaving a net increase in egg weight of 3.18 g as compared with the observed increase of 2.9 g. (Table 4.) Intermediate values may be interpolated. It probably would not be fair to extrapolate beyond the range of the protein percentages used

in the present experiment, because it is probable that the efficiency of feed containing increased increments of protein in the diet increases according to a hyperbolic curve rather than a straight line. Linear regression gives a fair fit only because the range of values is fairly narrow.

The other net correlations may be evaluated in the same manner. It should be noted that although the net correlation between percentage of protein in the diet and each of the several factors in egg production is in no case high, the total effect of increasing protein is very important.

The multiple correlations existing between the several factors studied are given in Table 11.

TABLE 11.—*Multiple correlation coefficients of the factors in egg production*

| Factor No. | Factors in egg production | Multiple correlations |
|---|---|---|
| $r_{1.234}$ | Egg weight with percentage of protein, body weight, and intensity | +0.492±0.0285 |
| $r_{2.134}$ | Percentage of protein with egg weight, body weight, and intensity | +.442± .0303 |
| $r_{3.124}$ | Live weight with egg weight, percentage of protein, and intensity | +.519± .0297 |
| $r_{4.123}$ | Intensity with egg weight, percentage of protein, and body weight | +.239± .0354 |
| $r_{5.234}$ | Yolk weight with percentage of protein, body weight, and intensity | +.300± .0342 |
| $r_{6.2345}$ | Albumen weight with percentage of protein, body weight, intensity, and yolk weight. | +.388± .0318 |

The values for $r$, the multiple correlation coefficient, indicate that percentage of protein in the diet, body weight, and intensity accounted for approximately 20 per cent of the variations present in egg weight. The remainder of the variation must be attributed to differences in quality of the protein supplements used, the inherent variability of the birds, and other causes unknown to the writers. The other coefficients may be interpreted in a similar manner.

### EFFECT OF DIET ON HATCHABILITY

During each month of the present experiment, except May and August, eggs were incubated. It was possible, therefore, to measure the relationship between intensity of production throughout the year and hatchability, as well as between average intensity and average hatchability for each hen. Table 12 gives the zero-order correlations between hatchability and percentage of protein and intensity.

TABLE 12.—*Zero-order correlations between hatchability, percentage of protein, and intensity*

| Factor No. | Egg-production factor | Correlation between factors indicated | |
|---|---|---|---|
| | | 2 | 3 |
| 1 | Percentage of protein | +0.226±0.0347 | −0.039±0.0365 |
| 2 | Intensity | | +.165± .0363 |
| 3 | Hatchability | | |

The correlation between percentage of protein in the diet and intensity of egg production has been considered and found to be significant. The coefficient of correlation between percentage of protein in the diet and hatchability, $r = -0.039 \pm 0.0365$, is negative and

not significant. The coefficient of correlation between average intensity and hatchability, $r = +0.165 \pm 0.0363$, is very low but significant. The writers treated these zero-order correlations by the method of partial correlation. Percentage of protein correlated with intensity, with hatchability held constant, ($r_{12.3}$) is not a logical relationship, for hatchability could not affect either of the other two factors. It became apparent that there is no significant relation between percentage of protein in the diet and average hatchability because the coefficient of net correlation, $r = -0.083 \pm 0.0364$, is not significant. The coefficient of net correlation between intensity and hatchability, $r = +0.179 \pm 0.0363$, is low but probably significant. It is likely that this latter correlation would be higher in a flock on a uniform diet or in a flock on diets containing successively larger percentages of the same protein concentrate, for it is shown that the effects of the several kinds of concentrate on hatchability bear no relation to their percentage of protein content.

If intensive laying depletes the hen's store of substances essential for the formation of hatchable eggs, or tends to do so, as Heuser (*9*) supposed, there should be a negative correlation between current production and hatchability. This does not necessarily require a negative correlation between average intensity and average hatchability, for a hen might alternate periods of very intense production with periods of broodiness or other nonlaying periods. She would thus have a low average intensity but high current intensity for the periods of actual egg production.

The hatchability of all individual clutches of eggs incubated in the present experiment was correlated with the percentage of production of the hens producing them for the month in which they were produced. The value for this correlation is $r = +0.197 \pm 0.0154$. This correlation is low but very significant, being more than 10 times its probable error. However, since the data used were obtained from incubations throughout the year, it might show only that the seasonal conditions conducive to high egg production furnish the most favorable climatic conditions for the collection, storage, and incubation of eggs, without necessarily indicating inherent differences in hatchability in the eggs themselves. To find out whether this was true, hatchability for March and for April was correlated with intensity of production for the same months. This correlation is $r = +0.225 \pm 0.0274$; it is more than eight times its probable error and is significant. This shows that even on the low-protein diets on which the hatchability was fairly good and the average intensity low, hatchability was best for the eggs of the hens laying most heavily. The correlation between current production and hatchability for a flock on a uniform diet would probably be higher. The coefficient of correlation between current hatchability and current intensity for diet 8 for the entire period of the experiment is $r = +0.298 \pm 0.0415$. It seems safe to conclude that hens able to lay eggs at a high rate on a particular diet will, in general, produce a higher precentage of hatchable eggs than those able to lay only at a low rate on the same diet.

### EFFECT OF DIET ON FERTILITY

Table 13 shows the effect of the diets on the fertility of the eggs produced and includes comparative data on the hatchability of fertile eggs. In fertility of eggs the greatest difference between diet 9

and any of the other diets, 5.81 ± 1.89, was found between diet 9 and diet 2. This is barely three times its probable error. Eggs from birds on diets 1, 2, and 8 were significantly higher in fertility than eggs from birds on diet 6. Table 13 shows that no diet consisting of feedstuffs of vegetable origin alone gave higher fertility than any diet containing an animal-protein concentrate, though the eggs from birds on diets 7 and 4 were practically equal in fertility.

TABLE 13.—*Effect of the various diets on the fertility of eggs and on hatchability of fertile eggs produced*

| Diet. No. | Eggs set | Fertile eggs | Differences in fertility of eggs from birds on indicated diet and on diet 9 | Differences in fertility of eggs from birds on indicated diet and on diet 6 | Hatchability of fertile eggs | Differences in hatchability of eggs from birds on indicated diet and on diet 9 | Differences in hatchability of eggs from birds on indicated diet and on diet 8 | Hatchability of total eggs (deduction made for breakage, infertility, and mortality) | Potential chicks per hen per day | Potential chicks per kilogram feed eaten |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Number* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Number* | *Number* |
| 1 | 736 | 96. 6±0. 528 | +4. 68±2. 14 | +7. 22±1. 69 | 67. 0±1. 96 | +2. 0±3. 19 | −6. 1±2. 66 | 50. 2 | 0. 271 | 2. 01 |
| 2 | 657 | 97. 7± . 544 | +5. 81±2. 14 | +8. 35±1. 69 | 72. 1±2. 09 | +7. 1±3. 27 | −1. 0±2. 93 | 57. 6 | . 295 | 2. 16 |
| 3 | 648 | 94. 2±1. 10 | +2. 27±2. 12 | +4. 81±1. 93 | 62. 8±2. 08 | −2. 2±3. 68 | −10. 4±2. 75 | 45. 9 | . 237 | 1. 76 |
| 4 | 652 | 92. 5±1. 55 | +. 56±2. 39 | +3. 10±2. 23 | 73. 4±1. 89 | +8. 4±3. 15 | +. 3±2. 61 | 49. 6 | . 228 | 1. 77 |
| 5 | 546 | 89. 4±1. 89 | −2. 49±2. 62 | ±0 ±2. 48 | 71. 6±1. 97 | +6. 6±3. 20 | −1. 6±2. 67 | 53. 3 | . 213 | 1. 68 |
| 6 | 669 | 89. 4±1. 60 | −2. 54±2. 42 | ..... | 61. 9±2. 32 | −3. 1±3. 43 | −11. 2±2. 94 | 40. 5 | . 191 | 1. 55 |
| 7 | 525 | 92. 4±1. 23 | +. 49±2. 19 | +3. 03±2. 02 | 56. 6±3. 47 | −8. 4±4. 29 | −16. 5±3. 91 | 45. 0 | . 196 | 1. 54 |
| 8 | 827 | 95. 0± . 86 | +3. 10±2. 01 | +5. 64±1. 82 | 73. 1±1. 80 | +8. 1±3. 10 | ..... | 59. 0 | . 329 | 2. 55 |
| 9 | 664 | 91. 9±1. 81 | ..... | +2. 54±2. 42 | 65. 0±2. 52 | ..... | −8. 1±3. 10 | 51. 0 | . 220 | 1. 85 |

None of the differences in hatchability of eggs from birds on diet 9 and those from birds on the other diets is significant, but the eggs from birds on diets 3, 6, and 7 show significantly lower hatchability than those from birds on diet 8. Diets 2, 4, 5, and 8 gave a distinctly superior hatchability of fertile eggs.

Table 13 shows that diets 1, 2, and 8 are more efficient for chick production than the others. Diet 7 was notably poor.

### EFFECT OF DIET ON EMBRYONIC MORTALITY AND TIME OF DEATH

Figure 3 gives the average mortality of each day for all eggs from birds receiving vegetable diets as compared with that for eggs from birds receiving animal protein. The curve for embryos from hens on vegetable diets had a peak in mortality during the second week of incubation which was not present in the curve for embryos from hens receiving animal protein.

The condition described by Dunn (*6*, *7*) and Landauer (*16*) as chondrodystrophy is shown by the embryo on the right in Figure 4. This embryo died at about the eleventh day of incubation; a normal embryo of that age is included for comparison. The chief points of difference are the shortened and recurved legs, the parrotlike beak, and the sparse down of the chondrodystrophic embryo. These embryos were very often edematous. In the present experiment most of them died during the second week of incubation but a few lived longer; none hatched. Table 14 gives the distribution of

chondrodystrophic embryos among the eggs from birds on the several diets.

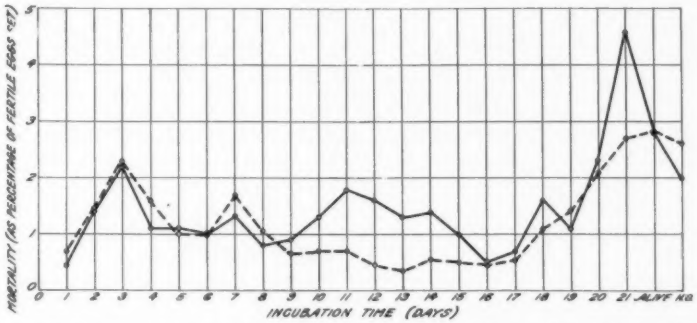


FIGURE 3.—Average percentage of mortality of embryos in eggs from hens on diets containing only vegetable feedstuffs (solid line) and in eggs from hens on diets containing animal-protein concentrate (broken line) as well. "H. O."=viable chicks helped out of shell on twenty-second day; "alive"=chicks alive in shell on twenty-second day

TABLE 14.—*Number and percentage of chondrodystrophic embryos from eggs produced on the different diets*

| Diet No. | Chondrodystrophic embryos | | Diet No. | Chondrodystrophic embryos | | Diet No. | Chondrodystrophic embryos | |
|---|---|---|---|---|---|---|---|---|
| | Number | Percentage of fertile eggs set | | Number | Percentage of fertile eggs set | | Number | Percentage of fertile eggs set |
| 1 | 4 | 0.56 | 4 | 2 | .34 | 7 | 25 | 5.25 |
| 2 | 2 | .31 | 5 | 7 | 1.40 | 8 | 3 | .30 |
| 3 | 2 | .33 | 6 | 30 | 5.03 | 9 | 16 | 2.61 |

Table 14 shows that the birds on diets containing animal-protein concentrates produced few chondrodystrophic embryos, whereas those on diets containing only feedstuffs of vegetable origin produced a relatively large number. The highest number from birds on a diet containing animal protein is 4, or 0.56 per cent of the number of fertile eggs set; the lowest number from birds on any diet consisting of vegetable feedstuffs is 7, or 1.4 per cent of the number of fertile eggs set. The eggs from birds on diets 6 and 7 had more than 5 per cent chondrodystrophic embryos. The average percentage of chondrodystrophic

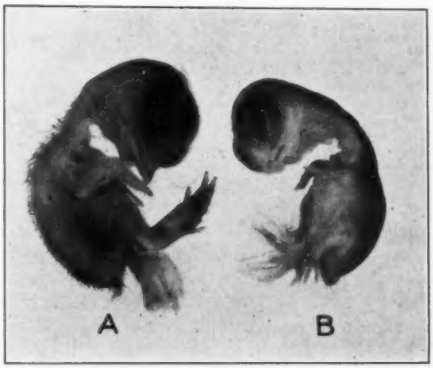


FIGURE 4.—Comparison of a chondrodystrophic embryo (B) with a normal embryo (A). Note the parrot-like beak and short, recurved legs of the former. Both embryos are about 11 days old

embryos in the eggs incubated from 34 hens which produced such embryos when fed vegetable diets was $21.98 \pm 2.53$, and from 12 hens fed the animal-protein diets the percentage was $6.3 \pm 0.788$. The difference between these averages is $15.68 \pm 2.65$; the difference is six times its probable error and shows conclusively that the tendency toward chondrodystrophy was greater among the embryos from the hens on the vegetable diets. The closeness of the incidence of chondrodystrophy in the progeny of these hens on the vegetable diets to the classical 3:1 Mendelian ratio given by a mating of heterozygous individuals involving a single recessive character, is without significance since the cockerels used were rotated from pen to pen.

Dunn (*7*) and Hutt and Greenwood (*10*) found that the occurrence of chondrodystrophy among the progeny of hens was not according to chance. This was true in the present experiment, as shown in Table 15, which gives the frequency distribution of chondrodystrophy among the embryos from the hens on vegetable diets compared with the frequencies of a Poisson series. The fit is very poor. Dunn (*7*) considered that his failure to increase the incidence of chondrodystrophy by inbreeding ruled out his original hypothesis of inheritance, but Hutt and Greenwood (*10*) considered it probable that hens bear a gene or genes for susceptibility to chondrodystrophy, the action of which may be augmented by lack of sunlight.

TABLE 15.—*Frequencies of chondrodystrophy among embryos from hens on vegetable diets, compared with frequencies of a Poisson series*

| Number of chondrodystrophic embryos | Observed frequency | Theoretical frequencies of Poisson series, using $nq=0.5$,[a] with average progeny | Theoretical frequencies with observed progenies | Number of chondrodystrophic embryos | Observed frequency | Theoretical frequencies of Poisson series, using $nq=0.5$,[a] with average progeny | Theoretical frequencies with observed progenies |
|---|---|---|---|---|---|---|---|
| 0 | 121 | 94.012305 | 94.092440 | 4 | 2 | 0.244900 | 0.824135 |
| 1 | 16 | 47.006075 | 42.578035 | 5 | 2 | .024490 | .214675 |
| 2 | 6 | 11.751480 | 13.195460 | 6 | 2 | .005115 | .000465 |
| 3 | 6 | 1.958580 | 4.094790 | | | | |

[a] The binomial is $\left(\frac{27}{28}+\frac{1}{28}\right)^{14} \times 155$. (The true value of the exponent, 14, is $13.93 \pm 0.799$ = average sample (progeny).)

Since the progenies varied in size, and the chances of occurrence of a particular number of chondrodystrophic embryos in a progeny are greatly affected by the size of the progeny, the values shown in the last column of Table 15 were calculated. The actual size of each progeny containing chondrodystrophic embryos was used in computing the probability of occurrence of the observed number of these embryos in that particular progeny. The average of the probabilities of all the progenies containing 0, 1, 2, 3, 4, 5, or 6 chondrodystrophic embryos was obtained and multiplied by 155, the total number of progenies, to obtain the values given in the table. These values give a somewhat better fit to the observed frequencies than do the frequencies obtained by using average progeny, but the fit is still too poor to permit the distribution to be attributed to chance.

According to the values in the third column of Table 15, a progeny containing 6 chondrodystrophic embryos would be expected once in

31,000 times. Actually, it occurred twice in 155 times; one of the progenies contained 8, the other 11 embryos.

Table 16 gives the seasonal distribution of chondrodystrophy among the progenies of hens on the vegetable diets that produced one or more chondrodystrophic embryos at some time during the experiment. Those from hens on the diets containing animal protein are not included on account of their small number.

TABLE 16.—*Seasonal distribution of chondrodystrophic embryos in eggs from hens on vegetable diets*

| Date of incubation | Fertile eggs set | Chondrodystrophic embryos | | | Date of incubation | Fertile eggs set | Chondrodystrophic embryos | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Number | Fertile eggs | Average of eggs of individual hens that produced such embryos | | | Number | Fertile eggs | Average of eggs of individual hens that produced such embryos |
| | *Number* | | *Per cent* | *Per cent* | | *Number* | | *Per cent* | *Per cent* |
| Oct. 2 | 12 | 1 | 8.3 | 5.0±3.37 | Feb. 19 | 53 | 5 | 9.4 | 11.1±3.45 |
| Oct. 30 | 31 | 6 | 19.7 | 32.6±9.99 | Mar. 17 | 56 | 15 | 26.8 | 33.5±5.57 |
| Nov. 27 | 30 | 2 | 6.7 | 5.6±2.58 | Apr. 16 | 70 | 23 | 32.9 | 38.6±5.71 |
| Dec. 28 | 46 | 6 | 13.0 | 17.2±5.61 | June 16 | 70 | 16 | 22.9 | 25.8±5.02 |
| Jan. 12 | 38 | 3 | 7.9 | 10 4±5.42 | July 15 | 34 | 0 | 0 | 0 |

Treated in this way, the data indicate that the peak of incidence falls in October and in the period from March to June, inclusive. However, the probable error of the October percentage is so great that it is by no means certain that the high value obtained is other than a chance fluctuation. The data are not incompatible with the hypothesis that these diets were incapable of supplying a sufficient quantity of some substance in the eggs essential to the production of normal embryos and that this lack was accentuated by the small amount of ultra-violet rays received through the winter and spring, so that the peak of incidence was reached during the season of highest production. The peak in April corresponds with the peak in Dunn's (*7*) data, but not with that in the data of Hutt and Greenwood (*10*), which occurred in January. Opposed to the hypothesis that lack of ultra-violet rays influenced the incidence of chondrodystrophy in the present experiment is the fact that the diets contained 2 per cent of cod-liver oil of standard potency. It seems more probable that the high incidence of chondrodystrophy in the embryos from the birds on vegetable diets is due to a deficiency of some substance other than vitamin D. The seasonal distribution described by Dunn (*7*) and Hutt and Greenwood (*10*) might be accounted for in other ways. The winter diet of their birds was certainly more restricted than the summer diet, if their birds were permitted range. Deficiency in some vitamin other than D, present in certain protein concentrates, or qualitative deficiency in the protein itself seems more likely.

## SUMMARY AND CONCLUSIONS

Single-Comb Rhode Island Red pullets and cockerels were fed diets containing various proteins of animal or vegetable origin to determine the effect of the proteins on egg production and hatchability. The

proteins comprised from 11.2 to 23.6 per cent of the diet. The basal diet consisted of a mixture of yellow corn meal, wheat bran, rolled oats, and alfalfa-leaf meal together with a mineral mixture and cod-liver oil. The various supplements to the diet were desiccated meat meal, crab-scrap meal, North Atlantic fish meal, dried buttermilk, dried-yeast preparation, soybean meal, cottonseed meal, and a combination of the meat meal, fish meal, and buttermilk.

The birds on the basal diet ate very well; they consumed practically as much feed as the birds on the supplemented diets. Current egg production was low; hatchability was fair.

Diet 1, meat-meal supplement, gave very good egg production and fair hatchability.

Diet 2, crab-scrap-meal supplement, was consumed readily and gave good egg production. The quantity of feed required to produce 1 g of egg was only a trifle higher than the quantity required when the birds received fish meal, and the hatchability of the eggs was better.

Diet 3, North Atlantic fish-meal supplement, gave good egg production, but the hatchability of the eggs produced was somewhat low.

Diet 4, buttermilk supplement, was excellent for winter egg production, egg weight, body weight, and hatchability of fertile eggs. Annual production was less than that of hens receiving diet 8, which was supplemented by meat meal, fish meal, and buttermilk.

The egg production on diet 5, yeast supplement, was not so good as that on the basal diet.

Diet 6, soybean-meal supplement, gave good egg production. Hatchability of fertile eggs was low, due in part to the fact that the incidence of chondrodystrophy in the eggs of certain hens was augmented.

Diet 7, cottonseed-meal supplement, delayed the beginning of egg production. It also increased the incidence of chondrodystrophy in the eggs of certain hens and gave low hatchability.

Diet 8, meat meal, fish meal, and buttermilk supplement, proved to be an excellent diet both for egg production and for hatchability.

Increasing the percentage of protein in the diet within the limits of 11.2 and 23.6 by the use of protein supplements of different origin augmented egg production (1) by increasing intensity of production; (2) by increasing egg weight through direct effect on yolk weight, and (3) indirectly, by increasing body weight and yolk weight, thereby increasing albumen weight.

Increasing protein level, within the limits stated, increased the quantity of egg produced per unit weight of feed eaten.

Increasing protein content decreased the efficiency of protein for egg production.

In general, during a given period, more eggs were hatchable from hens which laid intensely than from those which did not.

Diets containing proteins from vegetable sources only increased the incidence of chondrodystrophy in the embryos of hens likely to produce such embryos. Embryos in eggs from hens on such diets had a high second-week mortality.

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# THE INTERRELATIONS OF TWO HYMENOPTEROUS EGG PARASITES OF THE GIPSY MOTH, WITH NOTES ON THE LARVAL INSTARS OF EACH[1]

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## INTRODUCTION

Two imported hymenopterous egg parasites of the gipsy moth (*Porthetria dispar* L.) are established over a considerable part of the area of New England infested by that species, and it has been assumed that there was more or less competition between the two parasites when in the same area. It is the purpose of the writer to present some observations recorded during an attempt to determine the interrelationships of these parasites, *Anastatus disparis* Ruschka[3] and *Ooencyrtus kuvanae* (Howard),[3] when both occur in the same locality. In the course of the experiments some additional information concerning the larval instars of the parasites was obtained and is included in this paper.

*Anastatus disparis* (fig. 1) occurs in many parts of Europe and Japan. It was first colonized in this country in 1908 and since then has been liberated throughout most of the infested territory. *Ooencyrtus kuvanae* (fig. 2) is a native of Japan and was first colonized in this country in 1909. *A. disparis*, partly because of its wider distribution in the infested area of the New England States, is the more important parasite.

Previous publications dealing with the parasites of the gipsy moth contain accounts of these two species. Howard and Fiske[4] record instances of Anastatus larvae being parasitized by females of Ooencyrtus and of a generation of this parasite being carried through to maturity within the larvae of Anastatus. Crossman[5] states that in some instances larvae of both species have been found within the same host egg, but in such cases neither parasite has issued. It is also stated that it would seem from the observations made that Anastatus would suffer in an area where Ooencyrtus was abundant. Burgess and Crossman[6] state that these two parasites do not often conflict. These statements were suggested by observations made in connection with the importation, rearing, and colonization of these parasites, but the subject of the interrelations of the species has not previously been investigated in detail.

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[2] The writer is indebted to C. W. Collins, in charge of gipsy moth and brown-tail moth investigations of the Bureau of Entomology, for his cooperation in making these experiments possible. Acknowledgment is also made to C. F. W. Muesebeck for suggestions incorporated in the plan of the experiments, and to various workers on the staff for information taken from notes on file in the laboratory.

[3] Order Hymenopters, superfamily Chalcidoidea, family Encyrtidae.

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## SEASONAL-HISTORY NOTES ON THE HOST AND PARASITES

The greater part of the life of the gipsy moth is spent in the egg stage. The eggs, in a compact mass covered by the hairs from the abdomen of the female, are deposited during July and early August on

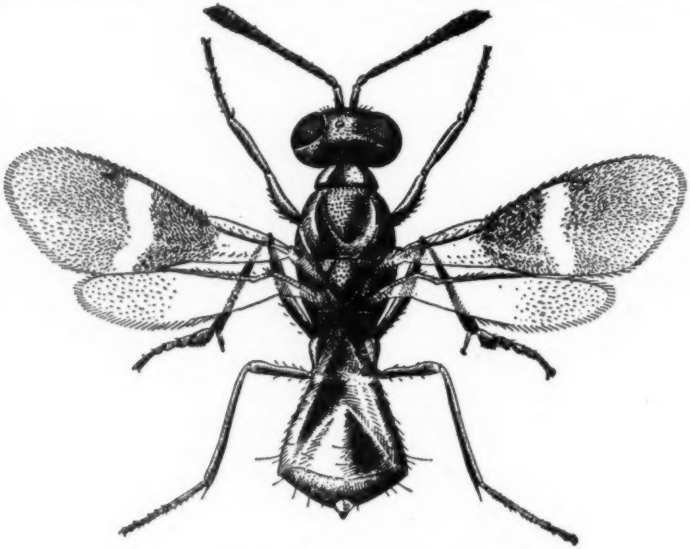

FIGURE 1.—*Anastatus disparis:* Adult female, × 22. (Howard)

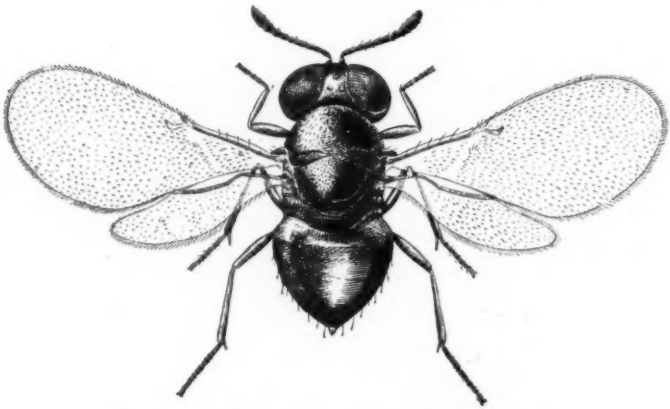

FIGURE 2.— *Ooencyrtus kuvanae:* Adult female, × 35. (Howard)

tree trunks, branches, stones, and, in fact, on almost any object in the immediate vicinity of the point where the female moth, which is incapable of flight, has emerged. Hatching usually occurs during the first two weeks of the following May. The young caterpillars grow

rather rapidly, and the larval life is completed in early July, when pupation occurs. The duration of the pupal period is about 11 days for females and 14 days for males. Oviposition occurs directly after the fertilization of the female moth.

*Ooencyrtus kuvanae* usually hibernates in the forest débris as an adult female. The females that survive the winter appear during the first warm days of April and have been observed ovipositing during the latter part of that month on bright warm days. Six weeks is required for the first generation to develop in overwintering eggs of the gipsy moth, the adults appearing during the last week of May and early June. Those of the second generation emerge during the first half of July. About three weeks is required for the development of the third generation, which is the first that is able to develop entirely on the newly deposited eggs of the host, emergence of the adults occurring during the first half of August. Under laboratory conditions a generation develops in 15 days. There is some overlapping of generations. Part of the adults of the first and second generations oviposit in dead and infertile eggs in the early spring, whereas others live until the new eggs are deposited and attack these. There is sufficient time for four complete generations and a partial fifth in New England.

*Anastatus disparis* hibernates as a full-fed larva within the egg of the gipsy moth and is rather well protected by the coating of hairs covering the egg mass. The adults may emerge as early as the middle of June or as late as the middle of August, but the majority are present in the field at the time of maximum oviposition by the gipsy moth, which normally occurs in July. The period from egg deposition to larval maturity is from 12 to 15 days. In some years, however, there is a partial second generation, which is of slight importance.[7] From this résumé it is apparent that the life history of *Anastatus disparis* coincides more closely with that of its host than does that of *Ooencyrtus kuvanae*. Furthermore, the hibernation of Anastatus is more successful than that of Ooencyrtus in the New England States because of its ability to withstand lower temperatures. It has been very difficult to bring adults of Ooencyrtus through the period of hibernation in laboratory experiments. Observations in the field indicate that the number of females living through the winter is very small.

## OUTLINE OF EXPERIMENTS

In an attempt to duplicate conditions as they might occur in the field, two parallel sets of experiments were conducted. In one set eggs were subjected first to *Anastatus disparis* and then to *Ooencyrtus kuvanae;* in the other they were subjected first to *O. kuvanae* and then to *A. disparis*. In the field no doubt there are more instances of *A. disparis* attacking the eggs first than of *O. kuvanae* attacking first, because of the greater numbers of Anastatus present at the time of egg deposition. An outline of the experiments conducted is given below.

(1) In experiment 1 a number of host eggs were subjected to parasitization by individual females of Anastatus. Each day a few eggs that had been attacked were placed with females of Ooencyrtus.

[7] The foregoing facts concerning the life histories of the host and parasites were taken from the previously cited papers of Burgess and Crossman, and of Crossman, with the exception of the duration of the pupal period of the host, which was taken from unpublished data collected by J. A. Millar of the gipsy-moth laboratory.

This was continued for 15 days to obtain a series of eggs containing Anastatus that had been attacked by Ooencyrtus at different periods of the egg and larval development of Anastatus.

(2) Experiment 2 was essentially like experiment 1, except that Ooencyrtus was allowed to attack the host eggs first, after which they were subjected to Anastatus. At the end of 15 days Ooencyrtus adults were issuing, which made it unnecessary to continue the experiments.

(3) One egg cluster from egg masses deposited by female moths on thin blocks of wood was fastened to the bottom of each of seven cages constructed of fine copper screening. These cages were cylindrical, 4 inches in depth and 5 inches in diameter, and had removable tin covers to which were fastened small metal handles; the cages themselves were fastened to wooden bases. Ten females of Anastatus were left in each cage for two days, after which all were removed. Ten females of Ooencyrtus were then introduced into one of the cages and left for two days. This was repeated with the other cages at 2-day intervals until all the cages had been treated in this manner. These cages and those used in experiments 4 and 5 were placed in the laboratory yard.

(4) Ten females of Ooencyrtus were placed in each of seven cages, of the foregoing type, and allowed to remain there for two days. The adults were then removed, and 10 females of Anastatus were placed in one of the cages and kept there for two days. The other cages were taken one at a time at 2-day intervals and treated in the same manner until all egg masses had been subjected to attack by both species.

(5) Five similar cages were used containing 10 females of each species caged together for 18 days.

(6) Two hundred and forty females of Anastatus were placed with 12 entire egg masses in a wooden box having a glass top. At the expiration of 2 days the Anastatus females were removed and 2 of the egg masses placed with 40 females of Ooencyrtus, where they were kept for 2 days. This was repeated at 2-day intervals with fresh parasites until all the masses had been subjected to Ooencyrtus.

(7) Experiment 7 was similar to experiment 6, except that Ooencyrtus was allowed to attack first, and the egg masses were then subjected to Anastatus.

(8) Experiment 8 was similar to experiment 6, except that the number of parasites used was decreased by one-half.

(9) Experiment 9 was similar to experiment 7 except that the number of parasites used was decreased by one-half.

This combination of experiments seemed comprehensive enough to give valuable information as to the behavior of each of the parasites in relation to the other. The first two experiments are the most valuable because the parasites were kept under observation at all times. The remainder of the experiments were conducted as a check upon the first two.

## PROCEDURE FOLLOWED IN THE EXPERIMENTS

In the first experiment the following method was used: One gipsy-moth egg was fastened to a small piece of paper by means of shellac and placed in a small glass vial (35 by 10 mm) with 1 female of

Anastatus. This was done to prevent interference between the females, such as had been noticed when a number of eggs were confined with several females. One man could watch about 50 of these vials with comparative ease, for the adults work rather slowly. When an egg was observed to have been attacked, it was removed and examined very carefully for the presence of a puncture made by the ovipositor of the female. Such a puncture would not necessarily signify the presence of a parasite egg (fig. 3), for the females often punctured the host eggs merely to provide openings at which to feed. Accordingly, it was necessary to subject entire egg masses to Anastatus, for the coat of hairs decreased the feeding by the adults. After the masses had been exposed for one day they were broken up and the eggs cleaned by rubbing them over cheesecloth stretched tightly over a wooden frame. The punctured eggs were then picked out for use in this experiment. For the first four days after attack by Anastatus it was impossible to determine definitely whether parasitism had taken place, but from that time on no egg was used which was not definitely known to contain an Anastatus larva. A few of these eggs were offered to Ooencyrtus each day, and as soon as they were attacked they were removed and examined for the presence of the egg of Ooencyrtus. This egg (fig. 4) has a "stalk" which extends

FIGURE 3.—Egg of *Anastatus disparis*, × 110. (Howard and Fiske)

FIGURE 4.—Egg of *Ooencyrtus kuvanae*, × 140

through the host egg sufficiently far to be easily recognized with a binocular microscope. This stalk, which is merely the end of the petiole of the egg, is definite evidence that Ooencyrtus has deposited an egg. By this method a series of host eggs was obtained containing eggs or larvae of Anastatus and which had then been subjected to Ooencyrtus while the former was in different stages of development.

The second experiment, while essentially like the first, differed slightly in method. Individual eggs were subjected to attack by Ooencyrtus and then submitted to Anastatus. It would have been better if entire egg masses had been submitted to Ooencyrtus and then to Anastatus and those eggs picked out that showed evidence of attack by both species. However, such a procedure would have involved sifting out and looking over so many eggs that it was impractical, and as Ooencyrtus readily attacked individual eggs no entire masses were used.

In all the remaining experiments the following method was employed: After the egg masses mentioned in the outline of work had been treated and the adults of Ooencyrtus had issued, the eggs were sifted from the clusters and counted. They were then separated into three groups—eggs from which Ooencyrtus adults had issued, eggs containing larvae of Anastatus, and unparasitized eggs. The adults of Ooencyrtus which emerged from these were not counted, for in many

cases two adults issue from one host egg. The eggs containing Anastatus larvae were examined for evidence of Ooencyrtus, as in the previous experiments.

## RESULTS OF THE EXPERIMENTS

An analysis of the information obtained from these experiments must take into account the direct observations made on the habits of the parasites as well as the results expressed in tabular form. If this is done the results of the experiments can be explained more clearly than would be possible if only the tabulations were used.

The results obtained in experiment 1 are shown in Table 1. A study of Table 1 reveals several significant facts. The more striking perhaps is that the only Ooencyrtus that reached maturity in the entire experiment were found in the eggs that were attacked during the period from a few hours to four days after attack by Anastatus. It was during this period that host eggs were used which were not definitely known to contain Anastatus, and it is doubtful whether any of the emerging Ooencyrtus developed within an egg that contained Anastatus.

TABLE 1.—*Results obtained from gipsy-moth eggs that were attacked first by Anastatus disparis, then subjected to Ooencyrtus kuvanae*

| Period between attack by Anastatus and attack by Ooencyrtus.....days.. | Less than 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Eggs showing evidence of attack by both species.....number.. | 10 | 10 | 9 | 11 | 17 | 9 | 10 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Eggs in which Anastatus developed to maturity....number... | 4 | 8 | 7 | 7 | 8 | 9 | 10 | 4 | 4 | 5 | 0 | 4 | 5 | 0 | 3 | 4 |
| Eggs in which Ooencyrtus developed to maturity.number.. | 6 | 2 | 2 | 4 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Another significant fact brought out by Table 1 is that the females of *Ooencyrtus kuvanae* were able to oviposit within an egg containing *Anastatus disparis* until it had reached the eighth day of its development. In some instances Ooencyrtus developed to the third instar, but when the Anastatus larva reached the last instar the only evidence of Ooencyrtus was the remnants of an incomplete anal shield. In other instances, owing to the irregularity of development, the Ooencyrtus females were unable to oviposit after six days. Observations showed this to be due to the mechanical action of the Anastatus larva. The larva is disturbed by the drilling operations of the Ooencyrtus female, and by the time the ovipositor enters the egg Anastatus is out of reach. The movements of the larva bring its head close to the opening made in the egg by the ovipositor of Ooencyrtus, and this places its body in a position where it can not be reached. Often the larva of Anastatus touched the ovipositor, which caused the female Ooencyrtus to leave the egg. A similar attempt was observed when Anastatus tried to oviposit in an egg containing Ooencyrtus, but with much less success on the part of Ooencyrtus, as the larva was somewhat handicapped by its attachment to the remains of its own eggshell, which restricted its movements. The ability of a larva of one parasite to move away from the ovipositor of another is due in part to the cautious manner in which the adult parasite feels

around within an egg before ovipositing, and also to the length of time required for a female to puncture the chorion of the egg.

From the results of this experiment it appears that Anastatus would suffer little in competition with Ooencyrtus. The presence of Ooencyrtus in the first part of the series is not surprising for the reason that it is impossible to make certain that host eggs contain Anastatus until five days after attack by this species. Aside from this short period the evidence is conclusive that Ooencyrtus does not displace the Anastatus larva.

The results of experiment 2 were rather disappointing, owing to the presence of a factor that could not be controlled. This was the feeding habit of Anastatus, previously mentioned. It rather upset the results of the experiment and made it difficult to draw satisfactory conclusions. By making due allowance for this habit, however, some information of value may be derived from this experiment. (Table 2.)

TABLE 2.—*Results obtained from gipsy-moth eggs that were attacked first by Ooencyrtus kuvanae, then subjected to Anastatus disparis*

| Period between attack by Ooencyrtus and attack by Anastatus.....days... | Less than 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | [a] 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Eggs showing evidence of attack by both species.....number.. | 9 | 11 | 10 | 10 | 10 | 10 | 8 | 9 | 15 | 22 | 12 | 14 | 15 | 5 | 6 | 10 |
| Eggs in which Ooencyrtus developed to maturity.....number.. | 7 | 7 | 10 | 10 | 9 | 4 | 2 | 4 | 5 | 8 | 7 | 10 | 11 | 1 | 1 | 8 |
| Eggs in which Anastatus developed to maturity.....number.. | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Eggs containing both species.....number.. | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ooencyrtus considered as killed by feeding of Anastatus adult.....number.. | 0 | 1 | 0 | 0 | 1 | 5 | 6 | 4 | 10 | 13 | 5 | 4 | 4 | 4 | 5 | 2 |

[a] At this time Ooencyrtus adults were issuing from host eggs.

Two facts stand out in Table 2: (1) The very small number of Anastatus that matured and (2) the large number of Ooencyrtus whose deaths were attributed to the feeding of the Anastatus females. Both of these circumstances resulted from the feeding habits of Anastatus, for it was noted throughout the experiment that the majority of the Anastatus females spent their time puncturing the host eggs in order to feed and in many cases did not oviposit. The death of a larva of Ooencyrtus noted in the third column of Table 2 was not caused by direct feeding by Anastatus but resulted from a reduction of available food, which prevented the larva from completing its growth. The later deaths, however, were caused by direct feeding of the adult Anastatus upon the body substance of the larvae or pupae of Ooencyrtus. In some instances it was possible to see the feeding tube constructed by the parasite extending from the body of the larva or pupa to the surface of the egg.

In all, four normal Anastatus larvae matured in this experiment. These were found in the eggs parasitized by Anastatus one day or less after Ooencyrtus had oviposited in them. In contrast with the condition in the first experiment it is certain that every egg used in this experiment had been oviposited in by Ooencyrtus before it was exposed to Anastatus. Therefore, in these four cases the egg or early-instar larva of Ooencyrtus must have been destroyed by the develop-

ing Anastatus larva. The remaining five Anastatus larvae occurring in this experiment were found in company with Ooencyrtus. In three instances the Ooencyrtus were larvae, and in the last instance there were two Ooencyrtus pupae within the one host egg with two larvae of Anastatus. In the instances where the egg contained both species all the Ooencyrtus larvae and pupae and four of the five Anastatus larvae were dead, the remaining one being barely alive.

The results in Table 2 indicate that it is possible for Anastatus to oviposit in an egg containing Ooencyrtus in any of its developmental stages, as some of the Ooencyrtus had pupated in nine days. There is some doubt whether either species would be able to complete its development if the attack by Anastatus occurred after the Ooencyrtus larva had reached the second instar, for in the eggs used in this experiment the second instar was reached on the third day, and no Anastatus matured in eggs attacked after two days had elapsed.

The importance of the feeding of the females of Anastatus would be over-emphasized if the figures in Table 2 were taken as an indication of the extent of its feeding under field conditions. The opportunity for the females to feed is much reduced on an entire egg mass because of the coating of hairs that covers a normal egg cluster. Egg masses will occasionally be found that are only partly covered, and in such cases considerable feeding by Anastatus might occur. If feeding did occur on the newly deposited eggs the development of the embryo would be prevented, as has been observed in some instances in the laboratory.

It is probable that experiment 1, in which the eggs were first attacked by *Anastatus disparis* and then by *Ooencyrtus kuvanae*, represents the situation more nearly as it occurs under field conditions, for it is probable that *A. disparis* is present in greater numbers at the time of deposition of gipsy-moth eggs than is *O. kuvanae*.

As already explained, the remaining experiments listed in the plan were conducted in order to check the results of the series in which individual eggs were used. As a whole the experiments were somewhat disappointing because the parasitism by Anastatus was lower than was expected. The adults did not work well in captivity under any conditions. The results, however, compare very well with those of the first two experiments. There is no indication of serious conflict between the two species, but it did appear that in a few instances Ooencyrtus oviposited in an egg containing Anastatus which resulted, as in the first experiment, in no development of Ooencyrtus. The results of these later experiments are not shown in tabular form because they present no information in addition to that furnished by the first two experiments.

Special efforts were made to duplicate an example, previously mentioned, of Ooencyrtus acting as an internal parasite of hibernating Anastatus larvae. Many attempts have been made in previous years to observe an incident of this sort, but without success. The writer, during these experiments, succeeded in obtaining reproduction of Ooencyrtus upon hibernating larvae of Anastatus by artificial means. Host eggs containing Anastatus larvae were placed for periods of from 4 to 15 minutes in a vial containing cotton that had been dipped in chloroform. These eggs were then subjected to attack by Ooencyrtus females, and oviposition was observed in some instances. The Anastatus larvae recovered from the effects of the

chloroform, but those larvae that had been attacked were killed by the developing Ooencyrtus larvae. It is possible that isolated instances of hyperparasitism occur, but only on larvae of Anastatus that are dead or dying at the time of attack by the females of Ooencyrtus.

### INTERPRETATION OF THE RESULTS

The results of the experiments here recorded indicate that it is advantageous to have both *Anastatus disparis* and *Ooencyrtus kuvanae* in the same locality. Theoretically it is possible that conditions might arise under which Ooencyrtus would be unable to reproduce itself, as, for instance, if Anastatus adults were present in such numbers as to utilize all the available host eggs first. If, on the other hand, Ooencyrtus had parasitized all the available eggs, it would be difficult for Anastatus to reproduce.

It is impossible, within reasonable limits, to ascertain the maximum percentage of host eggs that the varied sizes and shapes of the egg clusters make available to these parasites. Competition between the parasites would occur if the combined percentages of parasitism approached the limit of available host eggs. Field studies in the areas of New England where both these species are found do not indicate that there is any such conflict.

*Anastatus disparis* appears to be better suited than *Ooencyrtus kuvanae* to its host and to New England because its seasonal history parallels that of its host and because its hibernation is more successful in this area. Anastatus does not suffer from competition with Ooencyrtus, which succeeds in producing some parasitism in addition to that caused by Anastatus. The control exerted by the combined parasitism of the two species is greater than that which would be effected by either parasite working alone.

## LARVAL INSTARS OF THE PARASITES

The literature reviewed by the writer mentions only three larval instars for each of these two parasites. In the course of the investigations recorded in this paper five larval instars were observed in each species. Accordingly, the following descriptions are presented, and the mandibles of the five instars are figured.

### LARVAL INSTARS OF OOENCYRTUS KUVANAE

The first-instar larva of *Ooencyrtus kuvanae* is a small white maggot which, after hatching, is partly enveloped posteriorly with the remains of the eggshell. The body of the larva, indistinctly segmented beyond the fifth segment, tapers anteriorly from the fourth segment to the hemispherical head and posteriorly to the point of attachment to the hatched eggshell, where two open spiracles may be seen. After each molt the cast skin passes back to the posterior end of the larva and is retained, forming a shield covering the anal portion of the larva. The subsequent instars produce no great changes in the appearance of the larva except for the relatively shorter and stouter appearance it takes on with continued feeding. Just before pupation the last-instar larva becomes freed from the anal shield, which usually remains pressed against the inner surface of the eggshell of the host. There is no difficulty in distinguishing the larvae of either of these species on the basis of mandibles alone, but a simple means of identification of Ooencyrtus is the presence of the anal shield.

Figure 5 illustrates the differences between the larval mandibles of Ooencyrtus in the various instars.[8] In many instances it was possible to obtain the last four pairs of mandibles from one individual, but it was difficult to locate the mandibles of the first instar. The passage of the cast skins toward the posterior end of the larva takes place rather slowly, so it was possible to locate the mandibles of the second, third, and fourth instars in order on the body of the larva from the anterior end of the anal shield toward the head of the larva, which contained the last-instar mandibles. The first-instar mandibles were obtained from freshly hatched material.

The first-instar mandibles are rather peculiar in shape. It is impossible to determine with a compound microscope the limits of the bases of the mandibles because they are colorless and exceedingly small, measuring but 0.0038 mm in length. The points of the mandibles turn away from each other, differing in this respect from those of the succeeding instars. The average length of the mandibles measured for each instar was 0.0138 mm for the second instar, 0.0207 mm for the third, 0.0276 mm for the fourth, and 0.0483 mm for the last. There is a graduat transition from the second-instar mandible, which presents an acute angle between the tooth and the base, to the last-instar mandible with its broad sweeping inner margin. From colorless first-instar and second-instar mandibles the change is gradual to a deep yellow last-instar mandible.

### LARVAL INSTARS OF ANASTATUS DISPARIS

The first-instar larva of Anastatus is whitish and cylindrical, with a chitinized thimble-shaped head and a 13-segmented body which is broadest at the fourth or fifth segments and terminates in a bifurcate appendage. The body is rather densely clothed with spines and long sensory setae which are more numerous on the ventral portions. In the second instar the larva changes greatly in appearance. It loses the tegumentary spines and the bifurcate appendage, the head becomes more nearly spherical in outline, and the body appears relatively shorter and stouter. There is little change in the appearance of the larva from this instar through the following instars except for the continued thickening of the body, which in the last instar gives the larva a grublike appearance.

The larval mandibles of Anastatus (fig. 6) are much larger and heavier than those of Ooencyrtus. The mandibles of the first-instar larva, 0.0155 mm in length, are comma shaped, black at the distal end, becoming gradually lighter toward the base. The mandibles of the second-instar larva measure 0.0186 mm in length, those of the third instar 0.0276 mm, those of the fourth 0.0379 mm, and those of the hibernating or last-instar larva 0.0583 mm. The mandibles of the second-instar larva are colorless, whereas those of the third instar take on a light-brown color, which is confined mainly to the distal portion. The color of the mandibles increases in depth in the remaining instars, becoming a conspicuous reddish brown in the hibernating larva.

[8] The drawings of the mandibles of both species were made with the cover glass pressed down on a thin mount until all portions of the mandible, as far as possible, were in the same plane. All measurements were made in a straight line from the point of the mandible to the most distant visible point of the base.

## SUMMARY

In a large part of the New England area infested by the gipsy moth two imported hymenopterous parasites of the gipsy moth have become established. The first, *Anastatus disparis* Ruschka, is common in Europe and Japan, but the second, *Ooencyrtus kuvanae* (Howard), before its introduction into the United States was found only in Japan. From early observations made upon these parasites, it was suspected that there might be serious competition between them when present in the same locality. Since no attempts had been made to ascertain the interrelationships of these parasites, the writer conducted a series of experiments to determine the behavior of the two under conditions that would demonstrate competition if any were present. These


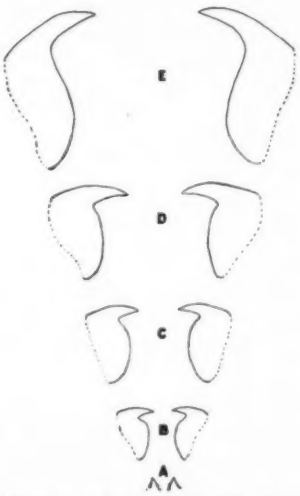


FIGURE 5.—Larval mandibles of *Ooencyrtus kuvanae:* A, B, C, D, E, First, second, third, fourth, and fifth instars, respectively

FIGURE 6.—Larval mandibles of *Anastatus disparis:* A, B, C, D, E, First, second, third, fourth, and fifth instars, respectively

experiments represented conditions as they occur in nature, that is, host eggs subjected to attack by either species were available for attack by the other at all stages in the development of the parasite which first attacks. Because Anastatus adults are probably more numerous at the time of deposition of host eggs, the greater number of instances of competition would be those in which Anastatus attacked first and Ooencyrtus attempted to reproduce upon those eggs already occupied by Anastatus in the egg or larval stages.

The experiments showed that there is little serious competition between the two parasites. Although Ooencyrtus females can attack a host egg containing Anastatus from the time that Anastatus is in the egg stage until it has become an early third-instar larva, it appears that Anastatus does not suffer from the attack. An egg containing Ooencyrtus throughout its egg, larval, and pupal life is to some extent

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susceptible to attack by Anastatus, but except for successful oviposition while Ooencyrtus is in the egg stage, which results in Anastatus developing, neither of the parasites succeeds in completing development. Since there is little conflict between the parasites in the same area under the conditions simulated in these experiments, the presence of the two may be considered advantageous, for parasitism by Ooencyrtus affords additional control to that effected by Anastatus. Anastatus, however, is the preferred parasite (1) because it is a single-generation parasite having a life history which coincides very closely with that of the host itself and (2) because it hibernates successfully in the area of New England infested by the gipsy moth. Ooencyrtus is multiple brooded and depends largely upon infertile and killed host eggs for the early spring generation. It hibernates as an adult, and suffers great losses during the winter months.

In the course of the investigations it was noted that, contrary to the general belief, there are five rather than three larval instars in the case of both the parasites. Short descriptions of the larvae are given, and the larval mandibles of both parasites are illustrated.

# CATALASE ACTIVITY AND RESPIRATION IN THE LEAVES OF GROWING BARLEY[1]

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## INTRODUCTION

There has been some difference of opinion as to whether there is an association between catalase activity and respiration. In 1910 Appleman (*1*)[2] noted a correlation between the two in the potato tuber and later (*2*) observed the same thing in potato tubers manipulated in various ways to modify respiration and in stem and seed ends of the same tuber. He found a similar correlation in stored sweet corn (*3*), and concluded that catalase activity is a fair index of the comparative intensity of respiration in the tissues of sweet corn and potato tubers. Morinaga (*11*), from studies of rice seedlings grown under differing conditions of aeration, concluded that there is a close positive connection between the two phenomena, and Burge (*4*) and Burge and Burge (*5*) are convinced, from studies of a number of organisms, that catalase activity is definitely associated with metabolism and with respiration in particular.

Others, however, have not obtained such convincing results. Crocker and Harrington (*6*) found a positive correlation between respiration and catalase activity in seeds of Johnson grass (*Sorghum halepensis* (L.) Pers.) and probably in those of *Avena fatua* L. but not in Amaranthus seeds. Rhine (*13*), working with germinating seeds, concluded that catalase activity is a measure of metabolism only when there is no rapid change in respiration. Lantz (*10*) did not find a close correlation between catalase activity and respiration in germinating corn of different chemical composition (high and low oil, high and low protein).

The writer has demonstrated widely different degrees of catalase activity in different parts of the same barley (*Hordeum distichon*) plant.[3] The relationship between catalase activity and respiration in leaves of different age is shown in this paper.

## MATERIALS AND METHODS

Plants of pure-line Hannchen barley, C. I.[4] 531, growing in a greenhouse at the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D. C.), were tagged for identification. A daily growth record of each leaf, based on measurements from the soil surface to the leaf tips, was kept for several days before the experiment. For each experiment three plants were selected, the homologous leaves of

[1] Received for publication Mar. 25, 1932; issued February, 1933.

[2] Reference is made by number (italic) to Literature Cited, p. 40.

[3] Unpublished data.

[4] C. I. denotes accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

which had made nearly equal growth. In these plants the developing spikes were small and still inclosed in the twelfth or boot leaf. From the first plant the six youngest leaves were removed, quickly weighed, and dried to constant weight at 80° C. at a pressure of approximately 6 cm of mercury. The second plant was laid away in a moist towel until the respiration run was started, when its leaves were individually tested for catalase activity. The leaves of the third plant were used for measuring the rate of respiration.

The respiration chamber was an Erlenmeyer flask of 125 c c capacity fitted with a 2-hole rubber stopper through which two glass tubes were passed, the upper ends projecting about 2 cm above the stopper. One of the tubes extended to within 2 cm of the bottom of the flask and the other only through the stopper. To the external ends of these glass tubes were fitted short pieces of rubber tubing with pinchcocks. The leaf to be investigated was loosely rolled on the finger, tied with a small cord, and suspended in the flask by slipping the end of the cord under a rubber band around the long glass tube. The chambers were set up in series with alternating blanks. The train was swept free of carbon dioxide, and 10 c c of nearly saturated barium hydroxide was introduced into each chamber through the hole stoppered by the short tube. The flasks were then shaken gently in a rocker shaker in the dark. In experiment 1, shaking was continued for 21 hours, and in experiment 2, for 23 hours. Temperatures ranged from about 24° to about 27° C. Blanks and respiration chambers were titrated alternately through the hole in the stopper, due precaution being used to prevent admission of atmospheric carbon dioxide. Titrations were made with 0.1362 normal hydrochloric acid, with phenolphthalein as an indicator. After the respiration run, the leaves were dried to constant weight and respiration rates were determined on both wet and dry weight bases. The slight error introduced by exposure to light and consequent assimilation during titration could not be determined.

Catalase determinations were made on leaves of the second plant by using the apparatus previously reported (*12*). Each leaf was triturated to a thin paste with washed sand, powdered calcium carbonate, and a small quantity of water. Enough water was added to make a 1 : 50 dilution based upon the green weight of leaf tissue. Two cubic centimeters of the suspension (equivalent to 0.04 g of green tissue) was then shaken with 3 c c of hydrogen peroxide in experiment 1 and with 2 c c in experiment 2. The volume of oxygen liberated for a dry-matter content of 0.008 g was calculated by using the average percentage of dry matter in homologous leaves of the other two plants.

## EXPERIMENTAL DATA

The respiration rates which are based on dry matter in the leaves after the run probably are somewhat high. (Table 1.) Homologous leaves were reasonably similar in growth rate, amount of dead tip tissue, and size of sheath and blade, and the data should be comparable.

The youngest leaf gave off carbon dioxide most rapidly. The quantity given off by the next older leaf was much smaller and dropped much more gradually to the fourth youngest. Carbon dioxide

evolution possibly was due in part to oxidation of dead tissues in the two oldest leaves. This factor may account for variability in that part of the curves.

TABLE 1.—*Respiration and catalase activity in homologous leaves from similar plants of Hannchen barley*

| Leaf No. | Respiration as $CO_2$ given off per hour per gram of— | | Catalase activity as $O_2$ evolved in 5 minutes— | |
|---|---|---|---|---|
| | Green weight | Dry weight | For 0.04 g green tissue | For 0.008 g dry tissue |
| | *Mg* | *Mg* | *Cc* | *Cc* |
| **EXPERIMENT 1** | | | | |
| 10 | 0.446 | 4.108 | 4.89 | 9.31 |
| 9 | .302 | 3.036 | 7.24 | 14.51 |
| 8 | .294 | 2.801 | 8.26 | 14.75 |
| 7 | .262 | 2.627 | 7.42 | 13.26 |
| 6 | .227 | 2.683 | 6.77 | 13.35 |
| 5 | .264 | 2.585 | 4.51 | 7.82 |
| **EXPERIMENT 2** | | | | |
| 12 | 0.584 | 5.043 | 5.37 | 8.92 |
| 11 | .338 | 2.873 | 11.74 | 18.67 |
| 10 | .309 | 2.403 | 14.80 | 22.81 |
| 9 | .283 | 2.266 | 13.22 | 21.45 |
| 8 | .285 | 2.587 | 12.27 | 21.15 |
| 7 | .243 | 2.471 | 10.52 | 19.77 |

In both experiments catalase activity was low in the youngest, most rapidly elongating leaf, but was much greater in the second youngest, which was elongating at about one-third the rate of the youngest, and was still greater in the next older. During senescence—indicated by greater length of dead leaf tip—catalase activity dropped off rapidly. Figure 1 gives a direct comparison between the respiration and catalase activity of homologous leaves on the two pairs of plants.

## DISCUSSION

Hover and Gustafson (*8, p. 39*) state that "as the leaves of corn, sorghum, wheat, and oats increase in age there is a decrease in rate of respiration; but that as the leaves become still older (past about middle age) the rate gradually increases." Their data, however, show this conclusion to be fully justified only in the case of sorghum. In their experiments the oldest corn leaves at time of pollination showed a higher rate of respiration than the youngest; in oats both plants used showed a rise with age, and in one wheat plant the youngest leaf showed a lower rate than did the next older, whereas in the other wheat plant used the situation was reversed.

In the present experiments it is assumed that the quantity of carbon dioxide given off is a measure of the respiration of the living tissues in the plant part. The results show a relatively high respiration rate for the youngest leaf of barley. There is a sharp decline in the curve to the next older leaf, after which the decrease in successively older leaves is much less, and at the fourth youngest leaf an approximate level is reached. This leaf had finished growth in length a week or

more before respiration was determined and had died back about 2 cm at the tip. Leaves still older had still more dead tissue, and the shape and irregularities of the respiration curve probably can be explained

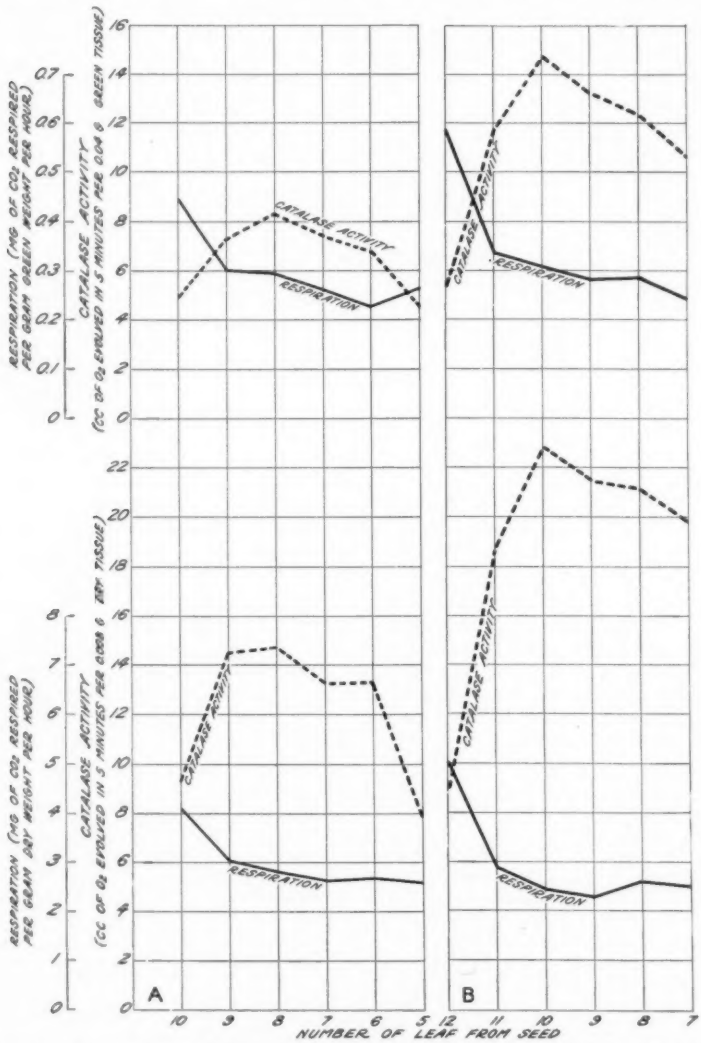
RESPIRATION (MG OF CO2 RESPIRED PER GRAM GREEN WEIGHT PER HOUR)
CATALASE ACTIVITY (CC OF O2 EVOLVED IN 5 MINUTES PER GRAM GREEN TISSUE)
CATALASE ACTIVITY
RESPIRATION
RESPIRATION (MG OF CO2 RESPIRED PER GRAM DRY WEIGHT PER HOUR)
CATALASE ACTIVITY (CC OF O2 EVOLVED IN 5 MINUTES PER GRAM DRY TISSUE)
A
B
NUMBER OF LEAF FROM SEED

FIGURE 1.—Respiration and catalase activity in homologous leaves from similar plants of Hannchen barley: A, Experiment 1; B, experiment 2

in part by the accumulation as age progresses of nonrespiring structural and senescent tissues. Rate of respiration, highest in the young leaf, decreased rapidly with advancing age to a point where it probably remained constant on the basis of living tissue.

Knott (*9*) found the youngest and oldest leaves of spinach and celery usually low in catalase activity as compared with those of intermediate age. Ezell and Crist (*7*) found a significantly negative correlation between catalase activity and plant size in lettuce but not in radish or in spinach.

In the experiments herein described catalase activity was relatively low in the youngest leaf of barley but increased in successively older leaves up to maturity. It then fell off rapidly with increasing necrosis of the oldest leaves.

On comparing catalase activity and respiration (fig. 1) a negative correlation is found between the two phenomena for the three youngest leaves. In the older leaves the curves are irregular and the relationships less definite, since catalase activity appears to decrease as living material decreases in the tissues, and apparent respiration possibly includes the production of carbon dioxide by oxidation of dead tissues.

Since in these experiments the only correlation discernible is negative, and since positive correlations have been found in stored potato tubers and sweet corn (*1*, *2*, *3*), in germinating seeds (*6*, *14*), and in rice seedlings (*6*), it seems highly probable that in no case is the relationship causal.

The nature of these results, contrary to those of most investigators, raises the question of the significance of catalase activity. The writer has assumed that the total catalase activity of the sample can be measured by prescribed methods. This assumption is true only if the agency responsible is a by-product of metabolism or if it is some part of the metabolic machinery which remains relatively stable and is not used up in the process. If either of these hypotheses is correct it must be concluded from the contradictory nature of the experimental data available from this and other studies that there is no causal relation between respiration and catalase activity.

On the other hand, the definition of catalase or catalase activity includes nothing but its reaction with hydrogen peroxide, which is the sole basis of its detection and determination. If this reaction occurs in normal tissues it is impossible to make an accurate determination of the agent producing it by shaking a tissue suspension with hydrogen peroxide, since the results then will be produced by the unused portion and will indicate only the excess of catalase manufactured over that utilized. It is conceivable that respiration and total catalase produced are positively correlated. In that case, in order to explain the results of these experiments it would be necessary to assume that in barley leaves high respiration is associated with a proportionately greater consumption of the catalase-activating agency, leaving a determinable surplus actually smaller in amount than when respiration is low.

## SUMMARY

The rate of respiration was high in young barley leaves and decreased in proportion to the rapidity with which the leaves matured.

Catalase activity was low in young barley leaves and increased to a maximum at about the time of leaf maturity, after which it decreased with decrease of living material in the tissues.

A negative correlation was found between respiration rate and catalase activity in homologous younger leaves of comparable barley

plants. In older leaves relationships were indefinite, probably masked by increasing amounts of structural material and necrotic tissues.

Since, contrary to the results of other investigators, a negative correlation was found between catalase activity and respiration, it is highly probable that any correlation is fortuitous.

Current methods of determination probably can measure total catalase activity only if that phenomenon is a by-product of metabolism or has a function in the tissues other than the breaking down of hydrogen peroxide.

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# A METHOD FOR DETERMINING THE QUANTITY OF MINERAL OIL RETAINED BY LEAF SURFACES AFTER SPRAYING [1]

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## INTRODUCTION

Perhaps the most important factor to be considered in the study of oil emulsions as insecticides is the amount of oil deposited upon foliage and insects. The quantity of oil deposited on a unit area of leaf surface depends upon the concentration of oil in the emulsion, the hardness of the water used for dilution, the concentration of the emulsifier, the method of application, the time of spraying, and many uncontrollable factors which only roughly regulate the effectiveness of the preparation. Hitherto no accurate figures have been available which would indicate what amounts of oil are required to kill either trees or insects, though it is agreed that the mineral-oil constituent left behind after spraying is the toxic agent. This paper gives the results of experiments conducted to develop a satisfactory method for the estimation of oil deposited upon foliage by spray emulsions.

## PRELIMINARY TESTS

English,[2] working with the leaves of Satsuma orange trees (*Citrus nobilis unshiu*), recently reported a method which is stated to be satisfactory for the determination of the oil retained upon plant surfaces. In this method, however, the natural oils and waxes that were extracted from the leaves along with the mineral oil that had been applied amounted to about 30 per cent of the total, so that subtraction of the volume of these natural substances was necessary in order to ascertain the amount of mineral oil deposited during spraying.

The first tests in the investigation reported here were made to determine the practicability of arriving at the quantity of oil retained by camphor leaves by finding the difference in the weights of individual leaves before and after spraying. Five leaves of camphor (*Cinnamomum camphora*) were detached from a garden tree at about 9 a. m. on June 13 and weighed at intervals to determine the losses due to evaporation. Table 1 gives the results.

The preliminary tests (Table 1) showed that the leaves lost from 14 to 26 per cent of their weight, owing to evaporation of moisture, in about eight hours. The conclusion was drawn that for this investigation the method of difference in weight of leaves could not be used, since the losses were of the same order of magnitude as the actual amounts of oil which might adhere to the surfaces.

[1] Received for publication Apr. 6, 1932; issued February, 1933.

[2] ENGLISH, L. L. METHOD FOR DETERMINING THE QUANTITY OF OIL RETAINED BY CITRUS FOLIAGE AFTER SPRAYING. Jour. Agr. Research 41 : 131–133. 1930.

TABLE 1.—*Loss in weight of camphor leaves by evaporation*

| Leaf No. | Initial weight | | Weight at end of 1 hour | | Weight at end of 2 hours | | Weight at end of 4 hours | | Weight at end of 8 hours | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Gram* | *Per cent* | *Gram* | *Per cent* | *Gram* | *Per cent* | *Gram* | *Per cent* | *Gram* | *Per cent* |
| 1 | 0.5034 | 100 | 0.4958 | 98.49 | 0.4859 | 96.52 | 0.4664 | 92.65 | 0.4206 | 83.55 |
| 2 | .4090 | 100 | .3934 | 96.19 | .3775 | 92.30 | .3536 | 86.45 | .3023 | 73.91 |
| 3[a] | .4450 | 100 | .4329 | 97.28 | .4185 | 94.04 | .3955 | 88.88 | .3419 | 76.83 |
| 4[a] | .5819 | 100 | .5691 | 97.80 | .5515 | 94.78 | .5218 | 89.67 | .4663 | 80.13 |
| 5[a] | .5078 | 100 | .5018 | 98.82 | .4938 | 97.24 | .4759 | 93.72 | .4358 | 85.82 |

[a] Broken end dipped in paraffin.

Analyses were then made to obtain information concerning the variation and the proportion of waxes or natural oils on camphor leaves. The determination of natural wax was made as follows: Samples, each consisting of 100 disks, were cut by a specially constructed die, similar to that described by Ginsburg,[3] from leaves gathered at random. The diameter of the disks was 30 mm. The disks were placed in 200 c c Erlenmeyer flasks, and 50 c c of ether was added. The flasks and contents were so rotated that the disks were washed thoroughly, but no liquid was lost. The first washing was filtered into a 125 c c Erlenmeyer flask. The extraction was repeated twice with two 25 c c portions of ether. After the last ether extraction the filter paper was washed repeatedly with 10 c c portions of ether to remove the wax left on it. The extractions and washings were evaporated to dryness under reduced pressure on a water bath, care being taken to prevent the liquid from reaching the cork stopper. The residue after evaporation was redissolved in 25 c c of ether and filtered again into a 50 c c flask which had been previously dried and weighed. In all cases in which a transfer of ether solution was made from one flask to another, the original container was washed out at least three times with pure ether, and the mouth was wiped with filter paper after each washing. The filter papers used for this purpose were placed in the original flask. This procedure, which prevented the loss of the extracted substances by evaporation in pouring, was used throughout the work. The solution in the 50 c c flask was evaporated to dryness under reduced pressure on a water bath, and the flask was then removed, dried with a cloth while warm, and reweighed. The waxy residue was calculated by the difference in the weight of the 50 c c flask when empty and when containing the residue.

The following figures show the variation in the amount of natural wax on camphor leaves as determined by this method:

| Sample No. | Weight of wax | Sample No. | Weight of wax |
|---|---|---|---|
| | *Gram* | | *Gram* |
| 1 | 0.0413 | 6 | 0.0331 |
| 2 | .0362 | 7 | .0356 |
| 3 | .0333 | 8 | .0397 |
| 4 | .0391 | 9 | .0383 |
| 5 | .0352 | 10 | .0351 |

The maximum variation from the average was 12.6 per cent.

[3] GINSBURG, J. M. AN APPARATUS FOR OBTAINING MEASURED AREAS OF SPRAYED FOLIAGE FOR CHEMICAL ANALYSES. Jour. Agr. Research 36: 1007-1009, illus. 1928.

In view of the fact that oil would actually be present during the analyses which follow, it was thought advisable to determine also what reliance might be placed on the figures representing the natural wax when the leaves were extracted in the presence of mineral oil. Accordingly, weighed amounts of Marcol oil were added to the sample prior to extraction, and these known weights of oil were subtracted from the total ether extract to obtain the values representing the wax. The results are given in Table 2. A maximum variation of about 30 per cent from the mean was found. The residue of natural wax was solid at room temperature, and produced a solidified mass when mixed with even three times its weight of mineral oil. The presence of oil during the analysis tended to increase the differences in the values obtained for wax content of the samples.

TABLE 2.—*Wax extracted from camphor leaves with ether in the presence of oil*

| Sample No. | Weight of residue | Weight of oil added | Weight of wax by difference | Sample No. | Weight of residue | Weight of oil added | Weight of wax by difference |
|---|---|---|---|---|---|---|---|
| | *Gram* | *Gram* | *Gram* | | *Gram* | *Gram* | *Gram* |
| 11 | 0.0605 | 0.0164 | 0.0441 | 17 | 0.0972 | 0.0625 | 0.0347 |
| 12 | .0744 | .0348 | .0396 | 18 | .1101 | .0817 | .0284 |
| 13 | .0723 | .0334 | .0389 | 19 | .1313 | .1028 | .0285 |
| 14 | .0675 | .0291 | .0384 | 20 | .1465 | .1194 | .0272 |
| 15 | .0830 | .0463 | .0367 | 21 | .1573 | .1327 | .0246 |
| 16 | .0892 | .0521 | .0371 | 22 | .1753 | .1443 | .0310 |

The variation in amount of natural wax obtained when extraction was made with chloroform in place of ether solvent in the method described above may be seen from the following tabulation:

| Sample No. | Weight of wax | Sample No. | Weight of wax |
|---|---|---|---|
| | *Gram* | | *Gram* |
| 23 | 0.0939 | 27 | 0.0976 |
| 24 | .0955 | 28 | .0896 |
| 25 | .1188 | 29 | .0910 |
| 26 | .0916 | 30 | .1151 |

These amounts are of the same order of magnitude as the amount of oil which might be actually retained by the leaves, and the variation in wax content from sample to sample was proportionally large.

On the basis of such results it was deemed necessary to introduce into the analytical procedure steps which would bring about the removal of wax and permit the quantitative recovery of the pure mineral oil.

## DETERMINATION OF OIL RETAINED BY CAMPHOR LEAVES

The two additional steps introduced are contained in the following method: 100 disks of 30-mm diameter were cut from leaves picked at random to make a single sample. Weighed amounts of Marcol oil (about 100 mg) were added with the extraction ether to the different samples. Each sample was washed for 1 minute with 50 c c of ether, as in the preceding method for the determination of wax, and then washed twice with 25 c c portions of ether to remove the last traces

of oil. The ether extractions were poured into a 125 c c Erlenmeyer flask and evaporated to dryness without filtering. The residue was dissolved at room temperature in a mixture of ether and absolute alcohol (1:1). On standing, a clear solution resulted. This was cooled to −30° C. for 15 minutes or until waxy flakes precipitated. The precipitated wax together with undissolved dirt, etc., was then filtered through a suction funnel surrounded with a 22 per cent sodium chloride constant-temperature mixture maintained at about −20° by small pieces of solid carbon dioxide (fig. 1) into another clean 125 c c Erlenmeyer flask.[4] The precipitated wax caught on the cold filter was washed three times with 10 c c portions of alcohol-ether mixture previously cooled down to −30°. The clear filtrate and washings were evaporated to about 10 c c and transferred by means of a funnel to a previously calibrated Babcock skim-milk bottle.

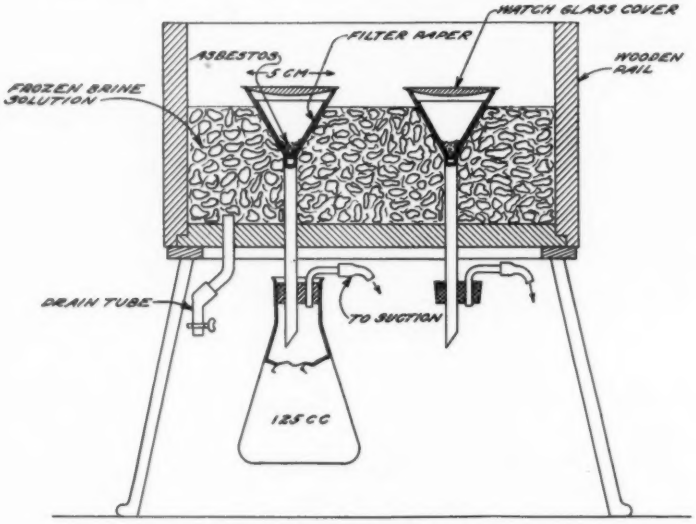


FIGURE 1.—Apparatus for cold filtration

The 125 c c Erlenmeyer flask was then washed three times with 5 c c portions of ether. The washings were poured into the Babcock bottle, care being taken to prevent loss by evaporation. By placing the forefinger over the mouth of the Babcock bottle and immersing the bottle in hot water the ether was made to evaporate through the capillary.

To the oily residue then in the Babcock bottle was added 6 c c (a few drops at first) of concentrated nitric acid (specific gravity 1.42), which initiated a violent reaction, partly destroying the unsaturated compounds and chlorophyll. The mixture, which consisted of acid, oil, and some partly destroyed organic matter, was raised to the boiling point by immersing the bottle in boiling water. After several agitations by hand, and after the oily layer appeared to be clear, the bottle was

[4] For the method of determination of paraffin, see the following publication: HOLDE, D. THE EXAMINATION OF HYDROCARBON OILS, AND OF SAPONIFIABLE FATS AND WAXES. Authorized transl. from German ed. 5, by E. Mueller. Eng. ed. 2, p. 108-109. New York. 1922.

filled to the neck with concentrated nitric acid and then centrifuged. The length of the column of oil was measured at room temperature by means of calipers equipped with a vernier permitting measurements to the nearest 0.1 mm. The skim-milk bottles of different sizes were calibrated by introducing mercury into the capillaries, measuring the length of the mercury column, recording the temperature of the air in the vicinity of the bottle, and calculating the volume per millimeter of length of each capillary. The specific gravity of the oil, which was determined to be 0.8447 at 15.5° C., was corrected 0.00061 per degree between 15.5° and room temperature, before converting the volume of oil measured into mass. The oil was clear and ran freely, but in some cases about 1 or 2 mm of solid matter not completely destroyed by the nitric acid came up into the capillaries. In such cases this wax or solid matter was measured as oil, since better results were so obtained. The oil used in all analyses was known to contain 97 per cent unsulphonatable matter. Blank tests showed, however, that the oil was not attacked by concentrated nitric acid under the conditions of these experiments, and correction for loss in oil due to the action of hot nitric acid was not necessary. Determinations of the oil in blank samples (containing no leaf waxes) agreed with the weights of oil taken, within 0.1 per cent, which was about the limit of error in observation, showing the calibrations also to be exact.

Table 3 shows the results obtained by this method in the determination of nine samples of oil that had been added to nine samples of camphor leaves. The error is comparatively large in the first four samples because the technic was not yet perfected.

TABLE 3.—*Recovery of oil previously added to samples of camphor leaves*

(100 disks per sample, 3 cm diameter)

FIRST SET

| Sample No. | Length of oil column | Volume per millimeter of capillary | Weight of oil added | Calculated weight of oil recovered | Error |
|---|---|---|---|---|---|
| | *Mm* | *Cc* | *Gram* | *Gram* | *Per cent* |
| 31 | 76.1 | 0.001552 | 0.1004 | 0.0995 | −0.89 |
| 32 | 89.0 | .001477 | .1121 | .1105 | −1.43 |
| 33 | 57.7 | .001616 | .0803 | .0784 | −2.36 |
| 34 | 77.8 | .001552 | .0998 | .1017 | +1.90 |

SECOND SET

| | | | | | |
|---|---|---|---|---|---|
| 35 | 59.5 | 0.001616 | 0.0805 | 0.0806 | +0.12 |
| 36 | 75.3 | .001577 | .1000 | .0995 | −.50 |
| 37 | 81.3 | .001609 | .1107 | .1102 | −.45 |
| 38 | 76.3 | .001552 | .1004 | .1003 | −.10 |
| 39 | 89.0 | .001477 | .1121 | .1105 | −1.43 |

## DETERMINATION OF OIL RETAINED BY PECAN LEAVES

That the method outlined above is also applicable to the determination of oil retained by pecan (*Hicoria pecan*) foliage following spraying is shown by the results in Table 4. No changes in the camphor-leaf method of determination were made except to heat the Babcock bottles, after nitric acid was added, to a temperature slightly higher than that used in the determination of oil on camphor leaves, in

order to destroy completely the acid-soluble wax. The bottles were heated and agitated directly over an electric heater until the acid began to boil.

TABLE 4.—*Recovery of oil previously added to samples of pecan leaves*

(100 disks per sample, 3 cm diameter)

| Sample No. | Length of oil column | Volume per millimeter of capillary | Weight of oil added | Calculated weight of oil recovered | Error |
|---|---|---|---|---|---|
| | *Mm* | *Cc* | *Gram* | *Gram* | *Per cent* |
| 1 | 77.5 | 0.001616 | 0.1057 | 0.1051 | −0.57 |
| 2 | 66.2 | .001609 | .0891 | .0894 | +.34 |
| 3 | 66.0 | .001577 | .0871 | .0875 | +.46 |
| 4 | 84.5 | .001477 | .1052 | .1048 | −.38 |
| 5 | 70.5 | .001464 | .0876 | .0868 | −.91 |
| 6 | 63.5 | .001616 | .0875 | .0868 | −.80 |
| 7 | 70.5 | .001609 | .0963 | .0954 | −.93 |
| 8 | 56.6 | .001477 | .0698 | .0702 | +.57 |
| 9 | 78.1 | .001616 | .1056 | .1060 | +.38 |
| 10 | 57.0 | .001577 | .0755 | .0756 | +.13 |

## DETERMINATION OF OIL RETAINED BY SATSUMA ORANGE LEAVES

As the application of the preceding methods to the determination of oil retained on Satsuma orange leaves was not successful, modifications were necessary. In preliminary tests, about 15 per cent of the oil added to the samples could not be recovered, as it was retained upon the disks after extraction and also upon the cold filter with the precipitated waxes. The first difficulty was avoided by extracting the leaf disks four times with 50 c c portions of ether instead of with one 50 c c portion and then with two 25 c c portions. As a matter of fact, 25 c c would not completely cover the disks. Though it was feasible to pass the cold extractions from the pecan leaves and the extractions from the camphor leaves, in a 1:1 alcohol-ether solvent, directly through the cold filter without previously filtering out dirt, scales, and other residue insoluble in ether, the same impurities, in the case of orange leaves, had to be filtered out immediately after extraction. It was found that such foreign matter promoted the occlusion of mineral oil in the wax precipitated later. With these precautions the error was reduced to about 7 per cent, but the results were far from satisfactory.

In order further to determine the optimum conditions under which a complete separation of natural wax and oil could be attained with Satsuma orange leaves, several additional trials were made in which the composition of the solvent used in freezing out the waxes and the temperature of the freezing bath were varied. Table 5 shows the results from some of these tests.

The results of the trial tests showed that a ratio of 20 c c alcohol to 10 c c ether gave the most effective solvent in which to precipitate the wax regardless of the temperature of the freezing mixture, whereas the optimum temperature of the freezing mixture was between −6° and −10° C. Above this temperature wax insoluble in nitric acid passed through the filter, and below it oil was retained on the filter with wax. Constant-temperature freezing mixtures were obtained by mixing different proportions of brine with water, and constant temperature was maintained by the use of solid carbon dioxide.

TABLE 5.—*Recovery of oil previously added to samples of Satsuma orange leaves, using different alcohol-ether mixtures at different temperatures*

(75 disks per sample, 3 cm diameter)

| Sample No. | Volume of alcohol | Volume of ether | Temperature of precipitation | Weight of oil added | Calculated weight of oil recovered | Error |
|---|---|---|---|---|---|---|
| | *Cc* | *Cc* | °*C.* | *Gram* | *Gram* | *Per cent* |
| **FIRST SET** | | | | | | |
| 1 | 5 | 15 | −20 | 0.0835 | 0.0688 | −17.6 |
| 2 | 10 | 10 | −20 | .0655 | .0554 | −15.4 |
| 3 | 15 | 10 | −20 | .0680 | .0640 | −5.9 |
| 4 | 20 | 10 | −12 | .0701 | .0654 | −6.7 |
| 5 | 20 | 10 | −6 | .0665 | .0660 | −.75 |
| **SECOND SET** | | | | | | |
| 6 | 10 | 15 | −20 | .0698 | .0640 | −8.31 |
| 7 | 15 | 10 | −20 | .0647 | .0627 | −3.09 |
| 8 | 20 | 5 | −20 | .0679 | .0634 | −6.63 |
| **THIRD SET** | | | | | | |
| 9 | 20 | 10 | −8 | .0630 | .0629 | −.15 |
| 10 | 20 | 10 | −8 | .0641 | .0635 | −.94 |
| 11 | 20 | 10 | −8 | .0652 | .0643 | −1.38 |

The order of magnitude of error involved in the determination of oil on Satsuma orange leaves under the optimum conditions may be seen from Table 6. Because it was impossible to recover all the oil, a correction of 1.5 per cent has been added. In general this correction gave better values. It would therefore be necessary in the analysis of unknown samples to add such a correction to the results. Even then a maximum error of 2.5 per cent is possible.

TABLE 6.—*Recovery of oil previously added to samples of Satsuma orange leaves under optimum conditions*

(75 disks per sample, 3 cm diameter)

| Sample No. | Length of oil column | Volume per millimeter of capillary | Weight of oil added | Calculated weight of oil recovered | Error |
|---|---|---|---|---|---|
| | *Mm* | *Cc* | *Gram* | *Gram* | *Per cent* |
| 12 | 44.0 | 0.001616 | 0.0617 | 0.0606 | −1.78 |
| 13 | 47.0 | .001609 | .0630 | .0645 | +2.38 |
| 14 | 52.0 | .001616 | .0706 | .0716 | +1.42 |
| 15 | 53.0 | .001477 | .0657 | .0667 | +1.52 |
| 16 | 50.4 | .001616 | .0702 | .0693 | −1.28 |
| 17 | 52.0 | .001578 | .0686 | .0700 | +2.04 |
| 18 | 47.7 | .001609 | .0666 | .0655 | −1.65 |
| 19 | 54.3 | .001477 | .0700 | .0683 | −2.43 |
| 20 | 50.5 | .001463 | .0641 | .0630 | −1.72 |
| 21 | 47.0 | .001609 | .0657 | .0645 | −1.83 |

## DISCUSSION

Before analyses of the type referred to are made, it is believed desirable to run a number of check samples in which the quantity of oil is known, in order to secure the correct working conditions and

technic. Naturally the methods, as given, are not applicable to the determination of unrefined oils (containing a relatively small proportion of unsulphonatable compounds) unless it can be shown that nitric acid either destroys a constant known percentage of the oil under consideration or does not attack the oil at all, as was the case in the present investigation. Although most oils used in spraying do contain appreciable amounts of unsaturated compounds, it is possible to select such refined oils in the study of the physical characteristics of emulsions as will not necessitate taking into account the action of nitric acid on the oil.

To illustrate one of the countless uses to which information concerning the retention of oil by trees may be applied, the following example is cited. It shows how the amount of oil deposited on camphor trees by sodium oleate emulsions containing different amounts of emulsifier changes with the concentration of the emulsifier. Six groups of nursery trees about 6 feet high, three trees to the group, were sprayed on August 10 with six different emulsions, trees of each group being sprayed with the same emulsion. In all six emulsions the concentration of Marcol mineral oil was constant (1.6 per cent), but the concentration of sodium oleate was different. Distilled water was used in the preparation and dilution. The soap was prepared directly from U. S. P. oleic acid and sodium hydroxide. A recently developed method [5] for spontaneously dispersing oil in water which obviates prolonged agitation was employed. After spraying, a sample of leaves was collected from each tree, three samples for each emulsion. The data in Table 7 are self-explanatory in respect to the number of leaf disks which composed the samples, the amount of soap contained in each emulsion, and the oil recovered.

TABLE 7.—*Oil retained on camphor leaves after spraying with emulsions of different concentrations*

| Group | Emulsion No. | Normality of soap $\times 10^4$ | Leaf disks per sample | Weight of oil found | Grams oil per $Cm^2$ [a] of leaf [b] $\times 10^6$ |
|---|---|---|---|---|---|
| | | | *Number* | *Gram* | |
| 1 | 1 | 29.8 | 100 | 0.0708 | 10.01 |
| | 2 | | | .0713 | 10.08 |
| | 3 | | | .0720 | 10.18 |
| 2 | 1 | 24.8 | 100 | .1030 | 14.56 |
| | 2 | | | .1010 | 14.28 |
| | 3 | | | .1075 | 15.20 |
| 3 | 1 | 17.38 | 75 | .1063 | 20.05 |
| | 2 | | | .1078 | 20.33 |
| | 3 | | | .1030 | 19.42 |
| 4 | 1 | 12.40 | 75 | .1143 | 21.55 |
| | 2 | | | .1085 | 20.46 |
| | 3 | | | .1228 | 23.16 |
| 5 | 1 | 7.44 | 50 | .0854 | 24.16 |
| | 2 | | | .0843 | 23.85 |
| | 3 | | | .0826 | 23.37 |
| 6 | 1 | 3.72 | 25 | .0428 | 24.21 |
| | 2 | | | .0432 | 24.43 |
| | 3 | | | .0418 | 23.65 |

[a] $Cm^2$ is the abbreviation for square centimeter recently adopted by the Style Manual for U. S. Government Printing Office.

[b] 1 $cm^2$ of leaf = 2 $cm^2$ actual leaf surface, therefore if concentration of oil on leaf surface is considered, the results in this column should be divided by 2.

[5] An account of this method has not yet been published. These emulsions are comparable in physical properties to emulsions prepared by the usual methods and possess several advantages over them.

From the data in Table 7 a curve has been plotted showing the variation in oil deposited with changing emulsifier concentration. (Fig. 2.)

## SUMMARY

The quantity of naturally occurring waxes extracted from camphor leaves by ether and by chloroform has been shown to vary in different samples with equal areas of leaf surface.

A method is given for the determination of the quantity of paraffin mineral oil retained by camphor leaves after spraying. The oil is recovered from the leaves in its original form, and the amount is measured volumetrically in calibrated Babcock skim-milk bottles.

By introducing certain variations the method is applicable to pecan leaves and Satsuma orange leaves. The maximum error in determining oil on pecan leaves was less than 1 per cent; on camphor leaves and Satsuma orange leaves it was less than 2.5 per cent.

An example is given illustrating the use of the method in estimating oil retained by camphor-tree foliage that had been sprayed with emulsions.

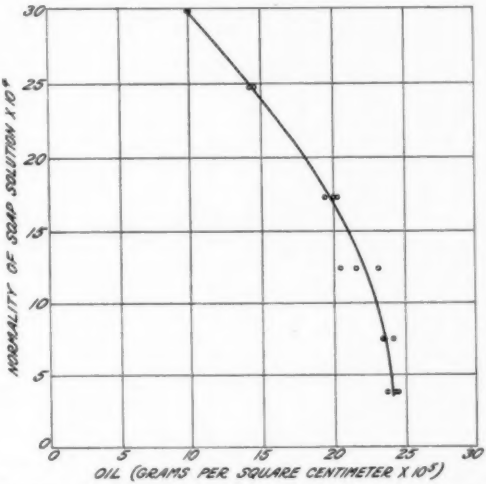


FIGURE 2.—Relation between emulsifier concentration and amount of oil deposited

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# DIFFERENCES IN THE AMINO ACID CONTENT OF THE CHIEF PROTEIN (GLYCININ) FROM SEEDS OF SEVERAL VARIETIES OF SOYBEAN [1]

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## INTRODUCTION

The enormously increased production of soybeans (*Soja max*) in the United States during recent years has emphasized their importance as an economic crop. A large proportion of the seeds produced are used as a source of oil, and the press cake which is obtained as a by-product is ground and sold as soybean meal.

Soybean meal is recognized as one of the best protein concentrates for stock feeding. It has been amply demonstrated, both by experimental studies in the laboratory and by the practical feeding of farm animals, that the proteins of the soybean have a high nutritive value.

Proteins differ widely in their nutritive value, depending chiefly on the quantities of certain amino acids which they yield on digestion. The chief proteins of some of our most important foodstuffs are deficient in one or more of the so-called nutritionally essential amino acids. Classic examples are zein from corn, deficient in lysine and tryptophane, and gliadin from wheat, deficient in lysine. Phaseolin, the chief protein of the navy bean, is deficient in cystine. It has been shown in previous publications from this division that the proteins of several other legume seeds, including the Lima bean, mung bean, velvetbean, adzuki bean, lentil, and cowpea, are likewise deficient in cystine. The soybean proteins, on the other hand, have been generally considered to contain adequate amounts of all the known essential amino acids. Mitchell and Smuts (*5*),[2] however, have recently reported that soybeans are rather low in cystine content.

The unusually wide range of differences in the characteristics of several soybean varieties has raised the question whether there may be a difference in the nutritional value of the protein of different varieties. In the case of the proteins of wheat, it is known, for instance, that gliadin and glutenin obtained from different varieties and classes of wheat have the same chemical composition. It is equally well known that different classes of wheat, and wheat grown in different environments, vary not only in their total protein content but also in their ratio of gliadin to glutenin. These two proteins, however, irrespective of the quantity or the ratio of each in the wheat, have always been found to have practically the same composition. Because of the lower nutritional value of gliadin, a variety of wheat having a high ratio of gliadin to glutenin would have a lower protein value than a variety which had a higher proportion of glutenin.

[1] Received for publication Apr. 6, 1932; issued February, 1933.
[2] Reference is made by number (italic) to Literature Cited, p. 55.

Similarly, soybeans, like most other seeds which have been investigated, contain several proteins which differ in chemical composition. Any difference found in the protein nutritional value of one variety over that of another would, therefore, be explainable on the assumption that these proteins occur in different proportions in the different soybean varieties. Information relative to possible differences in the nutritive value of various varieties from this standpoint would be of importance in the selection of varieties grown for the production of seed intended for use as a food or feed. The investigation described in this article was undertaken with the object of obtaining data on this question.

Protein concentrates as generally used for feed are not fed as the sole source of protein in the ration, but as supplements to other feeds having a lower protein content. It is important, therefore, that the proteins of the concentrates contain not merely enough of the essential amino acids to supply the nutritional requirements of the animal, but that they have even more, so that the excess may be available to compensate for possible deficiencies in the other protein-containing ingredients of the diet.

Osborne and Campbell (*7*) found that the chief protein of soybeans consists of a globulin which they named glycinin. This protein amounted to 16.6 per cent of the meal. Evidence was also found of the presence of a second globulin, which was more soluble than glycinin and which could be obtained from the solution from which glycinin had separated on dialysis. In addition to these two globulins there was isolated a legumelin, amounting to about 1.5 per cent of the seed, and a small quantity of proteose.

Glycinin, representing the globulin fraction obtained by dialyzing a saline extract of soybean meal, has been generally considered an individual protein. It constitutes the greater part of the total protein content of the soybean. Unpublished data recently obtained in this division indicate that glycinin thus prepared is not a single protein, but that it consists of at least two globulins. Furthermore, in a comparative study of the isoelectric points of different proteins, Csonka, Murphy, and Jones (*1*) found that glycinin was one of the few globulins studied which showed a deviation from the narrow range in isoelectric points characteristic of globulins as a class. This further supports the conclusion that glycinin is not an individual protein.

In the work here reported it was decided to limit the study to analyses of glycinin preparations similarly obtained from the different varieties. The ideal way would have been not to select any one protein or group of proteins, but to apply the analyses to the total protein content of the soybean. Amino acid determinations, however, can not be made even with approximate accuracy on the whole seed but must be made on the isolated proteins, therefore, the protein fraction selected for study was that which represents the greater part of the total protein of the soybean and which can most readily be isolated.

Of the three amino acids determined in the several glycinin preparations, cystine and tryptophane are known to be nutritionally essential. The percentages of these amino acids in the glycinin preparations can therefore serve as criteria of importance in a consideration of the relative nutritive value of the proteins of the different soybean varieties.

## MATERIAL AND METHODS

The soybeans used in this investigation were supplied by the division of forage crops and diseases of the Bureau of Plant Industry, and the varieties were selected on the merit of their widespread popularity among the soybean growers of the United States. The samples were obtained from localities in which the environmental conditions were known to be suited for the optimum growth and development of the varieties studied. The characteristics of these varieties are described in a recent publication by Morse (*6*).

The soybeans were ground to a meal, and the fat was removed by extraction with ether. The fat-free material was then extracted with 10 per cent sodium chloride solution. The saline extract was filtered by suction through a mat of filter-paper pulp, and the clear filtrate was dialyzed for 10 to 14 days. The glycinin which separated on dialysis was removed by centrifugation and washed several times with distilled water. It was finally dehydrated in the usual way with ethyl alcohol followed by ether.

Cystine, tyrosine, and tryptophane were determined in the different glycinin preparations by colorimetric methods. The method of Folin and Ciocalteu (*2*) was used for the tyrosine determination, that of Sullivan (*8*) for cystine, and May and Rose's method (*4*), with slight modification, for tryptophane. The maximum color development in the tryptophane determinations occurred after the casein standard and the glycinin sample had stood for five days at 38° C. The results of the analyses are given in Table 1.

TABLE 1.—*Tryptophane, cystine, tyrosine, and nitrogen content of glycinin prepared from different varieties of soybean seed expressed in percentages of moisture and ash-free protein*

| Variety | Color of seed | Tryptophane | Cystine | Tyrosine | Nitrogen | Variety | Color of seed | Tryptophane | Cystine | Tyrosine | Nitrogen |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Peking | Black | 2.03 | 0.81 | 3.94 | 17.19 | Mammoth Yellow | Straw yellow | 1.89 | 0.92 | 4.44 | 17.30 |
| Illini | Straw yellow | 2.84 | .74 | 4.55 | 17.74 | Haberlandt | do | 2.24 | .98 | 4.02 | 17.13 |
| A. K | do | 2.26 | 1.17 | 4.38 | 17.41 | Dunfield | do | 2.20 | .98 | 4.26 | 17.57 |
| Manchu | do | 2.36 | 1.45 | 4.36 | 17.28 | Dixie | do | 2.16 | .93 | 4.22 | 16.60 |
| Virginia | Brown | 2.28 | .95 | 4.49 | 17.43 | Chiquita | do | 1.94 | 1.46 | 4.31 | 17.16 |

## RESULTS OF ANALYSES

Although the differences found in the percentages of tryptophane and cystine in the glycinin preparations obtained from the soybean varieties studied are not great on the whole, some differences were found which are greater than can well be attributed to experimental error involved in the analytical procedures employed. Tryptophane, cystine, and tyrosine values determined on the same protein by the colorimetric methods used, particularly when the determinations are carried out by the same person under as nearly as possible identical conditions, may be expected to be within a limit of error of 2 to 3 per cent. The tryptophane values found range from 1.89 per cent for the Mammoth Yellow variety to 2.84 per cent for the Illini variety, a difference of nearly 1 per cent. The difference in tryptophane content between the Illini variety and the other varieties studied is great enough to be of nutritional significance.

The cystine values, on the other hand, range from 0.74 per cent for the Illini variety to 1.46 per cent for the Chiquita variety, a difference of 0.72 per cent. The Chiquita and Manchu varieties contain significantly higher percentages of cystine than the other varieties. It is of interest to note that of the varieties studied, the Illini variety, which contained the highest percentage of tryptophane, contained the lowest percentage of cystine, and that the Chiquita variety contained the highest percentage of cystine, but was among the lowest in tryptophane. The Peking and Dixie varieties were low in both cystine and tryptophane.

The cystine and tryptophane values found for the glycinin fraction of the soybeans studied are of significance from the standpoint of practical feeding. The Illini variety, because of its higher tryptophane content, should prove to be more valuable than the other varieties as a supplement to feeds, such as corn and oats, which are low in tryptophane. Feedstuffs which are deficient in cystine, such as cowpeas, lentils, and peas, should benefit more by supplementation with Chiquita and Manchu soybeans than with the Peking and Illini varieties, which contain only about one-half as much cystine.

In considering the application of the values found for these two nutritionallly essential amino acids to problems related to the feeding of soybeans, it must be remembered that the determinations were made on glycinin, and that, therefore, the values may not be quite typical of the total protein content of the seed. There are proteins other than glycinin present in soybeans concerning the composition of which we have little knowledge. It is possible that the tryptophane and cystine content of these proteins may be quite different from that of glycinin. In view of the fact, however, that these proteins are present in relatively small quantities, it is not probable that they affect much, one way or the other, the amino acid value of the whole soybean seed.

The glycinin preparations obtained from the different varieties agree closely in their nitrogen content with the exception of that of the Dixie and Illini varieties, the former containing the highest and the latter the lowest percentage of nitrogen. There is no apparent correlation between either the nitrogen and amino acid content of the different glycinin preparations or between the quantities of the three amino acids. As pointed out in a preceding paragraph, glycinin is probably not an individual protein but represents a mixture of two or more globulins. These globulins undoubtedly differ in their chemical composition. The variations found in the amino acid percentages obtained from the several varieties studied are then easily explainable on the ground of variations in the relative proportions in which the different globulins comprising the glycinin fractions are present in the different varieties of soybeans.

## DISCUSSION

In view of the fact that the different varieties of soybeans studied were not grown in the same localities, it is recognized that the differences found in the amino acid content of the glycinin preparations obtained from them are not necessarily characteristic of the varieties but may be due to the effect of external environmental conditions. Some varieties grow best in a warm climate, others do better in a cool climate.

The soybeans studied were grown in localities best suited for the growth and culture of the particular varieties. A large part of the total production of these varieties in the United States is located in those sections from which the samples analyzed were obtained. It is therefore believed that the results here presented are representative of these varieties as they are found on the market, and that, therefore, they have a greater value to the producer and consumer than if the different varieties had been grown in one place under identical environmental conditions.

The oil extracted from the soybeans used in this investigation has been studied by the Oil, Fat, and Wax Laboratory of this bureau, and the results are presented in another paper (*3*).

## SUMMARY

The results of colorimetric determinations of cystine, tryptophane, and tyrosine in glycinin preparations obtained from 10 different varieties of soybeans are reported.

The percentages of cystine, tryptophane, and tyrosine found for most of the varieties studied are in fairly close agreement. Differences between some varieties were found, however, which are greater than can well be attributed to experimental error involved in the analytical procedures employed. Tryptophane values ranged from 1.89 per cent for the Mammoth Yellow variety to 2.84 per cent for the Illini variety. The cystine values ranged from 0.74 per cent for the Illini variety to 1.46 per cent for the Chiquita variety.

The divergencies found in the amino acid content of the different glycinin preparations are explainable on the ground of variations in the relative proportions in which the different globulins comprising the glycinin fractions are present in the different varieties of soybeans.

The significance of the variations is discussed from the standpoint of nutritional value.

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# OIL CONTENT OF NINE VARIETIES OF SOYBEAN AND THE CHARACTERISTICS OF THE EXTRACTED OILS [1]

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It was considered of interest to determine the oil content of several varieties of soybean (*Soja max*) used in the protein investigation reported in another paper,[2] and to ascertain the more important chemical and physical characteristics of the oils extracted.

The oil content of the beans was determined in the usual manner by extraction with petroleum ether, but the oils upon which the characteristics were determined were obtained by ether extraction, because this solvent was preferable to the other for the preparation of the ground beans in the protein investigation. In the case of soybeans, however, there is little difference in the quantities of nonoil constituents extracted along with the oil by the use of either solvent. The ether was removed as completely as possible by distillation, and the remainder was removed by heating the oils in an oven in an atmosphere of carbon dioxide, at a temperature of about 115° C. until a constant weight was obtained. The results are given in Table 1.

TABLE 1.—*Chemical and physical properties of soybeans and their oils* [a]

CHEMICAL AND PHYSICAL CHARACTERISTICS OF OILS

| Item | Dunfield | Manchu | Haberlandt | Virginia | Chiquita | Mammoth Yellow | Peking | Illini | A. K. |
|---|---|---|---|---|---|---|---|---|---|
| Specific gravity—25°/25° C. | 0.9216 | 0.9218 | 0.9196 | 0.9198 | 0.9245 | 0.9224 | | | |
| Refractive index at 20° C. | 1.4748 | 1.4749 | 1.4745 | 1.4743 | 1.4763 | 1.4748 | 1.4763 | 1.4749 | 1.4756 |
| Acid value | | | | | | | 1.4 | 1.5 | 1.6 |
| Saponification value | 191.2 | 191.6 | 191.6 | 191.5 | 191.1 | 191.7 | 190.0 | 189.9 | 190.9 |
| Unsaponifiable matter—per cent | .73 | .88 | .80 | 1.08 | .85 | .85 | 1.10 | .86 | .89 |
| Iodine number (Hanus) | 131.4 | 131.0 | 131.6 | 127.8 | 140.7 | 129.4 | 141.4 | 131.3 | 132.6 |
| Thiocyanogen-iodine number | 84.1 | 82.2 | 82.2 | 81.4 | 87.0 | 83.4 | | | |
| Saturated acids—per cent | 12.6 | 11.9 | 12.5 | 12.5 | 11.7 | 11.7 | | | |
| Unsaturated acids—per cent | 80.4 | 80.9 | 80.2 | 80.1 | 81.0 | 80.8 | | | |
| Iodine number of unsaturated acids (calculated) | 162.6 | 161.5 | 162.1 | 157.8 | 172.4 | 159.1 | | | |

MOISTURE CONTENT AND OIL CONTENT OF BEANS

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Moisture—per cent | 5.87 | 5.62 | 5.40 | 6.46 | 7.24 | 6.93 | 5.61 | 7.30 | 6.00 |
| Oil—per cent | 18.86 | 18.12 | 18.37 | 19.13 | 16.79 | 15.61 | 17.98 | 19.28 | 21.85 |

[a] Analyses of beans and oil were made by R. S. McKinney and W. F. Baughman, oil, fat, and wax laboratory. The oils were extracted by ether so that the proteins could be investigated.

[1] Received for publication Apr. 6, 1932; issued February, 1933.

[2] CSONKA, F. A., and JONES, D. B. DIFFERENCES IN THE AMINO ACID CONTENT OF THE CHIEF PROTEIN (GLYCININ) FROM SEEDS OF SEVERAL VARIETIES OF SOYBEAN. Jour. Agr. Research 46: 15–55, 1933.

It will be observed from the table that the most striking differences found in the oils studied is in the iodine numbers and the thiocyanogen values. These differences are due to the variation in the proportions of oleic, linoleic, and linolenic acids in these oils. The oil from the seed of the Virginia variety has the lowest iodine number and the lowest refractive index, whereas the oils from the seed of the Chiquita and Peking varieties have the highest values for these characteristics.

Although the oil content of the seed from the different varieties shows a considerable range, the similarity in the proportions of saturated and unsaturated acids is noteworthy.

# RELATION OF WEATHER TO THE PREVALENCE OF WHEAT STEM RUST IN NEBRASKA[1]

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## INTRODUCTION

For the 10-year period 1921–1930 detailed observations were made on the appearance, subsequent development, and the prevalence of wheat stem rust (*Puccinia graminis tritici* (Pers.) Erikss. and Henn.) in Nebraska.[2]

This period is a rather short one for an analysis of the relation of weather to stem-rust development, in view of the conclusions reached by Lambert,[3] based on a study of stem rust during a 22-year period. He says that no evidence was obtained of any specific meteorological condition or set of conditions that always accompanies epidemics. "With so many interrelated factors involved, each epidemic appears to be a law unto itself. More precise information is needed, but the observer can not be cautioned too strongly against mistaking coincidences for causal relations." It can be assumed that certain factors must be contributing agents to the development of stem rust, since epidemics are the result of a progressive series or a combination of conditions favoring the rapid spread and development of stem rust.

The more important factors which may influence the development of stem rust of wheat in Nebraska are as follows: (1) The amount and regional distribution of initial inoculum reaching the wheat fields together with the time of its appearance; (2) the temperature and moisture favorable for the germination of the spores and subsequent infection; and (3) the amount and distribution of primary urediospores and the environmental factors suitable for the optimum development of the host and parasite, together with optimum conditions favorable for reinfection of the secondary and succeeding generations of urediospores. It is the purpose of this paper to discuss the influence of some of the above-mentioned factors on the prevalence of wheat stem rust and to point out the conditions under which rust epidemics may occur in Nebraska.

## RELATION OF SEASONAL VARIATION IN THE TIME AND LENGTH OF THE FRUITING PERIOD TO STEM-RUST EPIDEMICS

Before discussing the environmental factors influencing the development of stem rust, it might be well to point out the conditions that affect the time and length of the fruiting period of winter wheat. Observations over a 10-year period have shown that primary uredia

[1] Received for publication Apr. 26, 1932; issued February, 1933. Paper No. 120 of the Journal series of the Nebraska Agricultural Experiment Station.

[2] PELTIER, G. L. PHYSIOLOGIC FORMS OF WHEAT STEM RUST IN KANSAS AND NEBRASKA. Unpublished manuscript. —— and THIEL, A. F. STEM RUST IN NEBRASKA. PART I. GENERAL SURVEY OF SOURCES. PART II. IDENTIFICATION OF THE PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS FROM VARIOUS SOURCES. Nebr. Agr. Expt. St. Research Bul. 42, 40 p., illus. 1927.

[3] LAMBERT, E. B. THE RELATION OF WEATHER TO THE DEVELOPMENT OF STEM RUST IN THE MISSISSIPPI VALLEY. Phytopathology 19: 1-71, illus. 1929.

have never been found in the spring on winter wheat in Nebraska prior to the late-boot or early-heading stage. Thus, with the exception of the environmental factors necessary for initial infection, the variations in the time and length of the fruiting period may be one of the major factors in determining the development and prevalence of stem rust in Nebraska.

TABLE 1.—*Seasonal variation in the time and length of the fruiting period of Turkey Red winter wheat[a] during 28 years at Lincoln, Nebr*

| Year | Date of heading | Date of ripening | Length of fruiting period | Yield per acre |
|---|---|---|---|---|
| | | | *Days* | *Bushels* |
| 1903 | June 2 | July 11 | 39 | 32 |
| 1904 | June 4 | July 13 | 39 | 18 |
| 1905 | May 29 | July 2 | 34 | 21 |
| 1906 | May 28 | July 3 | 36 | 62 |
| 1907 | June 7 | July 8 | 31 | 52 |
| 1908 | May 29 | June 29 | 31 | 38 |
| 1909 | June 17 | July 13 | 26 | 27 |
| 1910 | May 31 | July 2 | 32 | 48 |
| 1911 | May 30 | June 23 | 24 | 51 |
| 1912 | May 31 | July 5 | 35 | 9 |
| 1913 | May 30 | June 28 | 29 | 50 |
| 1914 | June 1 | June 25 | 24 | 34 |
| 1915 | ....do | July 8 | 37 | 30 |
| 1916 | June 2 | July 7 | 35 | 38 |
| 1917 | June 17 | July 17 | 30 | 32 |
| 1918 | May 26 | June 27 | 32 | 19 |
| 1919 | June 3 | July 3 | 30 | 26 |
| 1920 | June 9 | July 7 | 28 | 41 |
| 1921 | May 26 | June 20 | 25 | 37 |
| 1922 | May 28 | June 24 | 27 | 31 |
| 1923 | June 6 | July 6 | 30 | 24 |
| 1924 | June 3 | ....do | 33 | 33 |
| 1925 | May 29 | June 23 | 25 | 15 |
| 1926 | May 28 | June 24 | 27 | 14 |
| 1927 | June 7 | July 6 | 29 | 37 |
| 1928 | May 29 | July 3 | 35 | 24 |
| 1929 | June 3 | ....do | 30 | 34 |
| 1930 | May 27 | June 30 | 34 | 43 |
| Average | June 2 | July 3 | 31 | 33 |

[a] Crop considered in head or ripe when approximately 80 per cent of the culms had reached the specified condition. These are composite results for ordinary Turkey Red wheat grown on the Nebraska Experiment Station farm, at Lincoln. The 1917 winter-wheat yield is the average for 3 fields of wheat that did not winterkill. The wheat in a fourth field, representing about one-third of the total acreage, winterkilled, and the field was replanted to corn. These data were supplied by Dr. T. A. Kiesselbach, agronomist, Nebraska Agricultural Experiment Station. The data prior to 1924 have been reported by him in the following publication: KIESSELBACH, T. A. WINTER WHEAT INVESTIGATIONS. Nebr. Agr. Expt. Sta. Research Bul. 31, 149 p., illus.

Table 1 shows the time of heading and ripening and the length of the intervening period for Turkey Red wheat for 28 years (1903–1930) at Lincoln, Nebr. There is a range of 22 days in the time of heading (May 26, in 1921, and June 17, in 1917). The average date of heading for the entire period is June 2. Likewise, there is a range of 27 days in the time of ripening (June 20, in 1921, and July 17, in 1917). The average date of ripening for the 28 years is July 3. The length of the fruiting period varies from 24 to 39 days, with a range of 15 days. The average length of the fruiting period is 31 days. As is to be expected, the yields obtained ranged from 9 bushels in 1912 to 62 bushels in 1906, with an average of 33 bushels. With such wide variations in the time of heading and ripening and the length of the fruiting period, it seems likely that the prevalence of stem rust from year to year would also vary greatly, because the environmental factors responsible for the seasonal variations in the development of

the wheat plant from heading to maturity would also influence the development and prevalence of stem rust.

To ascertain the influence of weather upon the length of the fruiting period, the mean temperature, amount of precipitation, and number of rainy days for the actual period for each of the 28 years are given in Table 2. It is apparent that with few exceptions there is a relation between mean temperatures and length of the fruiting period, the shorter periods having higher mean temperatures than the longer ones. Thus the average mean temperature for the period of 24 to 27 days is near 75° F., whereas for the period of 36 to 39 days it is about 69°, about 6° less. The direct influence of precipitation can not be determined because of its variability from year to year. The same may be said for the number of rainy days. There is a tendency, however, toward the occurrence of high rainfall and numerous rainy days along with the lower mean temperatures and extended periods of fruiting. There are, however, two exceptions to this statement—an extremely high rainfall occurred with a high mean temperature in 1914 and a low rainfall with a low mean temperature in 1903.

TABLE 2.—*Influence of weather on the length of the fruiting period of Turkey Red winter wheat at Lincoln, Nebr.*

| Year | Actual length of fruiting period | Mean temperature | Precipitation | Rainy days |
|---|---|---|---|---|
| | | LENGTH OF FRUITING PERIOD 24 TO 27 DAYS | | |
| | *Days* | *° F.* | *Inches* | *Number* |
| 1909 | 26 | 76.5 | 4.90 | 10 |
| 1911 | 24 | 77.4 | .56 | 6 |
| 1914 | 24 | 76.0 | 10.06 | 14 |
| 1921 | 25 | 75.0 | 4.40 | 9 |
| 1922 | 27 | 74.8 | 1.27 | 8 |
| 1925 | 25 | 75.6 | 6.15 | 12 |
| 1926 | 27 | 70.1 | 2.65 | 9 |
| Average | 25 | 75.1 | 4.28 | 10 |
| | | LENGTH OF FRUITING PERIOD 28 TO 31 DAYS | | |
| 1907 | 31 | 73.8 | 5.38 | 9 |
| 1908 | 31 | 68.4 | 11.33 | 20 |
| 1913 | 29 | 73.7 | 2.02 | 9 |
| 1917 | 30 | 74.7 | 3.50 | 6 |
| 1919 | 30 | 75.7 | 3.74 | 11 |
| 1920 | 28 | 75.2 | 2.10 | 10 |
| 1923 | 30 | 72.5 | .79 | 8 |
| 1927 | 29 | 72.4 | 3.08 | 7 |
| 1929 | 30 | 72.4 | .93 | 7 |
| Average | 30 | 73.2 | 3.65 | 10 |
| | | LENGTH OF FRUITING PERIOD 32 TO 35 DAYS | | |
| 1905 | 34 | 70.6 | 4.75 | 12 |
| 1910 | 32 | 72.3 | 2.57 | 7 |
| 1912 | 35 | 73.1 | 3.15 | 12 |
| 1916 | 35 | 70.5 | 3.14 | 11 |
| 1918 | 32 | 76.8 | 2.54 | 11 |
| 1924 | 33 | 69.1 | 4.22 | 16 |
| 1928 | 35 | 68.2 | 3.97 | 17 |
| 1930 | 34 | 69.7 | 3.39 | 9 |
| Average | 34 | 71.3 | 3.47 | 12 |

TABLE 2.—*Influence of weather on the length of the fruiting period of Turkey Red winter wheat at Lincoln, Nebr.*—Continued

LENGTH OF FRUITING PERIOD 36 TO 39 DAYS

| Year | Actual length of fruiting period | Mean temperature | Precipitation | Rainy days |
|---|---|---|---|---|
| | *Days* | *° F.* | *Inches* | *Number* |
| 1903 | 39 | 70.5 | 2.32 | 12 |
| 1904 | 39 | 69.2 | 6.85 | 21 |
| 1906 | 36 | 69.9 | 7.50 | 12 |
| 1915 | 37 | 67.4 | 5.12 | 18 |
| Average | 38 | 69.3 | 5.45 | 16 |

While it is true that the mean temperatures during the fruiting period determine to some extent the length of this period, it is of interest to know what the weather conditions were prior to and during the interval from heading to maturity. Therefore, the weather data for the months of May, June, and July for each of the 28 years, and the departures from normal are given in Table 3, grouped as in Table 2. The data show that where the length of the fruiting period was from 24 to 27 days, the mean temperatures, with few exceptions, were above normal in all three months, whereas the precipitation and number of rainy days were in general below normal. The year 1914 was exceptional, since an excess of rain occurred during each of the three months.

TABLE 3.—*Departures from normal of mean temperatures, amount of precipitation, and number of rainy days for the months of May, June, and July for a 28-year period at Lincoln, Nebr.*[a]

LENGTH OF FRUITING PERIOD 24 TO 27 DAYS

| Year | May | | | June | | | July | | | Total departure | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Temperature | Precipitation | Rainy days | Temperature | Precipitation | Rainy days | Temperature | Precipitation | Rainy days | Temperature | Precipitation | Rainy days |
| | *°F.* | *Inches* | *Number* | *°F.* | *Inches* | *Number* | *°F.* | *Inches* | *Number* | *°F.* | *Inches* | *Number* |
| 1909 | −1.3 | +1.55 | +1 | −0.1 | −1.24 | −1 | −0.3 | +1.02 | −1 | −1.7 | +1.33 | −1 |
| 1911 | +3.7 | −.13 | 0 | +7.3 | −3.76 | −5 | +1.7 | −2.02 | −4 | +12.7 | −5.91 | −9 |
| 1914 | +2.5 | +.12 | −4 | +3.8 | +6.39 | +6 | +2.7 | +.99 | −1 | +9.0 | +7.50 | +1 |
| 1921 | +3.6 | −.98 | −3 | +5.0 | +.11 | −2 | +2.3 | −.90 | +2 | +10.9 | −1.77 | −3 |
| 1922 | +2.1 | −.86 | +3 | +4.0 | −1.95 | +2 | −2.7 | +2.38 | +6 | +3.4 | −.43 | +11 |
| 1925 | −1.1 | −2.83 | −3 | +2.8 | +2.32 | +2 | +1.7 | −1.78 | −1 | +3.4 | −2.29 | −2 |
| 1926 | +4.5 | −1.13 | +1 | −.5 | −1.74 | −2 | +2.1 | −2.01 | +1 | +6.1 | −4.88 | 0 |
| Average | +2.0 | −.61 | 0 | +3.2 | +.02 | 0 | +1.1 | −.33 | 0 | +6.3 | −.92 | 0 |

LENGTH OF FRUITING PERIOD 28 TO 31 DAYS

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1907 | −5.5 | −0.80 | +1 | −2.0 | +1.93 | 0 | −0.1 | −0.19 | 0 | −7.6 | +0.85 | +1 |
| 1908 | −1.2 | +1.27 | +7 | −2.2 | +6.92 | +8 | −2.3 | +4.23 | +2 | −5.7 | +12.42 | +17 |
| 1913 | +1.3 | +1.94 | +1 | +2.6 | −2.05 | −1 | +4.5 | −1.90 | +1 | +8.4 | −2.01 | +1 |
| 1917 | −4.9 | −.09 | 0 | −1.7 | +1.71 | +3 | +1.9 | −3.29 | −4 | −4.7 | −1.67 | −1 |
| 1919 | −1.0 | −.25 | −2 | +1.6 | +.67 | +2 | +5.7 | −3.47 | −5 | +6.3 | −3.05 | −5 |
| 1920 | −.7 | −.31 | +3 | +1.6 | −2.27 | −4 | +.1 | −.05 | +1 | +1.0 | −2.63 | 0 |
| 1923 | −1.5 | −.77 | +1 | +.7 | +.93 | +1 | +2.3 | −1.97 | −3 | +1.5 | −1.81 | −1 |
| 1927 | −.5 | −2.51 | −2 | −1.8 | −.89 | −1 | −.1 | −1.97 | −1 | −2.4 | −5.37 | −4 |
| 1929 | −1.7 | −.80 | −2 | −1.0 | −2.46 | −2 | +.9 | +1.47 | 0 | −1.8 | −1.79 | −4 |
| Average | −1.7 | −.27 | +1 | −.2 | +.50 | 0 | +1.4 | −.79 | −1 | −.5 | −.56 | 0 |

[a] Climatic data in this and succeeding tables supplied by T. A. Blair, senior meteorologist, Weather Bureau, U. S. Department of Agriculture, Lincoln, Nebr.

TABLE 3.—*Departures from normal of mean temperatures, amount of precipitation, and number of rainy days for the months of May, June, and July for a 28-year period at Lincoln, Nebr.*—Continued.

LENGTH OF FRUITING PERIOD 32 TO 35 DAYS

| Year | May | | | June | | | July | | | Total departure | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Temperature | Precipitation | Rainy days | Temperature | Precipitation | Rainy days | Temperature | Precipitation | Rainy days | Temperature | Precipitation | Rainy days |
| | °F. | *Inches* | *Number* | °F. | *Inches* | *Number* | °F. | *Inches* | *Number* | °F. | *Inches* | *Number* |
| 1905 | −2.1 | +1.03 | +3 | +0.2 | −0.89 | −1 | −3.9 | −0.60 | +4 | −5.8 | −0.46 | +6 |
| 1910 | −4.5 | −.47 | +1 | +.6 | −2.23 | −5 | +.3 | −.82 | −1 | −3.6 | −3.52 | −5 |
| 1912 | +3.9 | −3.12 | −4 | −3.0 | −1.33 | 0 | +2.5 | −1.67 | +1 | +3.4 | −6.12 | −3 |
| 1916 | +.5 | −.60 | +1 | −3.6 | −1.21 | −1 | +5.6 | −2.56 | −3 | +2.5 | −4.37 | −3 |
| 1918 | +6.3 | −1.26 | −1 | +5.8 | −1.79 | −2 | +.9 | −1.34 | 0 | +13.0 | −4.39 | −3 |
| 1924 | −6.0 | −1.88 | 0 | −2.3 | .00 | +6 | −3.6 | +.81 | +2 | −11.9 | −1.07 | +8 |
| 1928 | +2.9 | −1.33 | −4 | −4.6 | −.35 | +6 | +.5 | +1.87 | +4 | −1.2 | +.19 | +6 |
| 1930 | −.3 | −.98 | +1 | −.2 | −1.37 | −3 | +6.3 | −2.79 | −3 | +5.8 | −5.14 | −5 |
| Average | +.1 | −1.08 | 0 | +.9 | −1.15 | 0 | +1.1 | −.89 | 0 | +.3 | −3.11 | 0 |

LENGTH OF FRUITING PERIOD 36 TO 39 DAYS

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1903 | −0.4 | +6.64 | +8 | −5.0 | −1.72 | 0 | −1.0 | −0.78 | −1 | −6.4 | +4.14 | +7 |
| 1904 | −.9 | +.12 | +2 | −3.6 | +.08 | +5 | −3.8 | +1.27 | +7 | −8.3 | +1.47 | +14 |
| 1906 | +1.7 | −2.55 | −3 | −1.0 | −.72 | −1 | −4.1 | +3.00 | 0 | −3.4 | −.27 | −4 |
| 1915 | −2.7 | +.69 | +3 | −4.2 | −.29 | +2 | −4.8 | +2.89 | +10 | −11.7 | +3.29 | +15 |
| Average | −.6 | +1.22 | +3 | −3.5 | −.66 | +1 | −3.4 | +1.60 | +4 | −7.5 | +2.16 | +8 |
| Normal | 61.7 | 4.08 | 11 | 71.4 | 4.32 | 11 | 76.5 | 3.85 | 9 | | | |

In years when the fruiting period was from 28 to 31 days, temperatures for the most part averaged somewhat below normal for May, near normal for June, and in the majority of years slightly above normal for July. In general, precipitation was below normal in May, slightly above normal in June, and below normal in July. On the whole, however, temperatures and precipitation for the three months were only slightly below normal. In 1908 there was a large excess of precipitation during all three months, and also a larger than normal number of rainy days.

When the fruiting period was from 32 to 35 days (with the exception of 1918, when temperatures were well above normal) the temperatures were near or slightly below normal for May and June and above normal for July. With few exceptions the precipitation was below normal, in fact more so than in the last-mentioned group.

In those years with a fruiting period of from 36 to 39 days, temperatures averaged below normal for all three months, while the rainfall was above normal for May and July and only slightly below for June. Here again there seems to be a tendency for high rainfall to be associated with low mean temperatures. A comparison of the data in Tables 2 and 3 reveals a relationship between the weather conditions prevailing during the fruiting period and for the 3-month period in any one year.

Lambert [4] points out that the years of rust epidemics in the spring wheat area were 1904, 1911, 1916, 1919, 1920, and 1923, whereas

[4] LAMBERT, E. B. Op. cit.

little or no rust occurred in 1907, 1909, 1910, 1912, 1913, 1915, 1918, and 1924. The years 1905, 1906, 1908, 1914, 1917, 1921, 1922, and 1925 he classifies as intermediate or indeterminate as far as rust was concerned. Nebraska records show that 1904, 1916, 1919, 1920, and 1923 (in western Nebraska) were epidemic years. The year 1927 should be classed as potentially an epidemic year, although the actual loss from stem rust in Nebraska was not large. In the remaining years, while the amount of rust varied, it was not severe enough to attract attention, and the actual losses were not estimable.

In all the years in which the fruiting period was between 24 to 27 days no estimable losses from rust occurred. With the exception of 1909, the average date of heading was May 29 and of ripening June 23. (Table 1.) While Lambert [5] includes 1911 as an epidemic year in the spring-wheat area, the length of the fruiting period at Lincoln was short (24 days). The plants were in head in late May and were ripe by June 23. The mean temperatures were high, and a large deficiency in moisture occurred, so that environmental conditions were also very unfavorable for rust development. As a group, these years can be classified as rust escaping, because of the short period from heading to ripening and also because the early maturity of the grain did not permit the development of many generations of urediospores, even though rust might have been present at the time of heading and optimum conditions for its development might have been at hand.

Three of the five epidemic years are included in the second group for which the fruiting period was from 28 to 31 days. In these years the mean temperatures for the period varied from 72.5° to 75.7° F., and in two years the precipitation and the number of rainy days favored rust development. In 1923 the rainfall was short, and in consequence there was not a great deal of stem rust in the vicinity of Lincoln. The wheat headed the first week in June, or shortly thereafter, and ripened the first week of July. During three other years in this group (1907, 1927, and 1929) the times of heading, maturity, and weather conditions were somewhat similar, and no epidemic occurred. In 1908, while temperatures were below normal and exceedingly high precipitation with many rainy days occurred, conditions favored the development of rust, and yet apparently very little developed. In 1908 and 1913 wheat headed early and ripened the latter part of June, while growth was abnormal in 1917 because of excessive winter killing.

The weather conditions and length of the fruiting period in 1916 did not differ in any important respect from the other years in this group (32 to 35 days fruiting period); therefore, some factor or combination of factors other than those mentioned was responsible for the epidemic in that year. With the exception of 1924 the wheat was in head by the first of June and ripened prior to the time at which wheat matured in 1916. A rather severe epidemic occurred in 1904, when there was an extended fruiting period, but with the exception of precipitation, the weather conditions in 1903 were very similar to those in 1904.

The data for the 28-year period indicate that stem rust will not assume epidemic proportions in eastern Nebraska during those years

[5] LAMBERT, E. B. Op. cit.

when winter wheat heads prior to, or during the first week in June and ripens before July 1. The length of the fruiting period does not permit many urediosporic generations,[6] and in most years weather conditions conducive to the rapid maturity of the wheat usually do not favor rust development. Rust epidemics have occurred during years when wheat did not head until June and matured in July, or when the length of the fruiting period was extended. There are years with extended fruiting periods, however, when the same weather conditions prevail as in years of wheat stem rust epidemic but when only small amounts of rust develop in Nebraska. Therefore, a study of the other factors influencing the development of stem rust was undertaken to determine the part played by some of these contributing factors in abetting or inhibiting rust development. The results of this study follow.

## AMOUNT, TIME OF APPEARANCE, AND GENERAL DISSEMINATION OF INITIAL INOCULUM

Observations made during 10 years show that the amount of local inoculum near infected barberry bushes has varied from year to year, depending primarily on the number of viable teliospores present, the weather conditions favoring their germination, and the subsequent infection of the barberries. Furthermore, in many instances, other races of stem rust have been found in the vicinity of infected barberry bushes. On the whole the limited amount of local initial inoculum near infected barberry bushes is decreasing as barberry eradication is carried on. This is especially true when the amount is contrasted with the widespread regional distribution of wind-blown urediospores from the South.

It is difficult to determine the amount of initial inoculum which reaches Nebraska each season. During certain years primary uredia are very hard to find, even after a search of several hours in wheat fields, whereas in other years they are not only readily found in any field but in large amounts over a wide area. Primary uredia were much more readily found in 1922, 1923, and 1927 than in the other years. Therefore the amount of initial inoculum which reaches Nebraska as wind-blown urediospores usually in early June, varies each year and may depend on the number of urediospores which survive the winter in the South and the conditions affecting their development and dissemination.

The time of appearance of initial inoculum also varies with the season, as has already been discussed by the writer.[7] In Table 4 the dates of the appearance of primary uredia near Lincoln, Nebr., are listed. During 6 of the 10 years primary uredia were found between June 7 and 9. In the remaining four years uredia were found May 30, June 12, and as late as June 18 and 20. The average date for the 10 years was June 10, and the range was 21 days, a period sufficiently long to account for great differences in the development and severity of stem rust from year to year.

[6] "Urediosporic generations" is used here for the want of a more inclusive term. It is true that from the time a uredium sheds its first spores until new uredia are subsequently produced, no increase in the amount of rust is possible. From this time onward, however, no such period exists, since both old and new uredia shed spores continuously until they are depleted. The progressive rate of increase is enormous from a very small number of primary uredia providing environmental factors are favorable.

[7] PELTIER, G. L. Op. cit.

By means of simultaneous surveys primary uredia have been found in occasional years at about the same time in south and northeast Nebraska. As a rule, however, primary uredia have been found from a few days to a week or more later in northeast Nebraska, and from one to two weeks later in the western part of the State. Thus, the amount and time of appearance of initial inoculum together with its general distribution vary from year to year. The exact amount of initial inoculum present in Nebraska each year, however, remains one of the undetermined factors.

TABLE 4.—*Weather conditions prevailing for a 2-week period prior to the appearance of primary uredia at Lincoln, Nebr., 1921 to 1930*

| Year | Date of first uredia | Beginning of 2-week period | Mean temperature for period | Amount of precipitation for period | Rainy days for period |
|---|---|---|---|---|---|
| | | | *°F.* | *Inches* | *Number* |
| 1921 | June 8 | May 25 | 71.7 | 3.94 | 6 |
| 1922 | May 30 | May 16 | 64.4 | 2.36 | 8 |
| 1923 | June 7 | May 24 | 69.6 | 4.70 | 5 |
| 1924 | June 20 | June 6 | 70.9 | 2.24 | 10 |
| 1925 | June 12 | May 29 | 75.3 | 1.41 | 6 |
| 1926 | June 8 | May 25 | 71.0 | .61 | 4 |
| 1927 | do | do | 63.0 | 1.16 | 5 |
| 1928 | June 18 | June 4 | 67.2 | 1.23 | 6 |
| 1929 | June 9 | May 26 | 66.1 | 3.36 | 7 |
| 1930 | do | do | 64.9 | .79 | 4 |

## WEATHER CONDITIONS FAVORING PRIMARY INFECTION

It has been pointed out that the time of appearance of primary uredia varies from year to year at Lincoln. In Table 4 the annual climatic data for two weeks prior to the finding of primary uredia are given. The weather conditions are an additional factor that varies from year to year. From the temperature standpoint, favorable conditions for a heavy primary infection occurred in 1921, 1923, 1924, 1926, and 1928. Temperatures slightly below the optimum prevailed in 1922, 1927, 1929, and 1930. The temperature in 1925 was too high for optimum infection. Sufficient moisture and rainy days occurred during all the years included in this study, with the possible exception of 1926 and 1930, for favorable infection. Apparently temperatures suitable for primary infection are more of a limiting factor than is moisture, in most years in Nebraska.

## RELATION OF THE TIME OF HEADING OF TURKEY RED WHEAT TO DATE OF APPEARANCE OF PRIMARY UREDIA AT LINCOLN

Table 5 shows that the appearance of primary uredia does not always coincide with the time of heading of winter wheat. In 1922, 1923, and 1927 primary uredia were found at about the time of heading, whereas in 1921, 1924, 1925, 1928, and 1930 uredia did not appear until about two weeks or more after the plants had headed. In the remaining two years rust did not appear until 6 and 11 days after heading. Thus the time of heading and the date of appearance of primary uredia have a direct bearing on the further development of rust. For example, in 1921, 1924, 1925, 1926, and 1928 only about

two weeks remained for further rust development. It must be borne in mind that wheat plants approaching maturity can not be infected with rust, so that the period of susceptibility to rust is really shorter than two weeks. In the other years from three weeks to four weeks remained for secondary and succeeding infections. In other words, in these years the time necessary for increasing urediosporic generations to a point sufficient to produce an epidemic was sufficient, provided weather conditions favorable for optimum infection and development of rust prevailed.

TABLE 5.—*Relation of the time of heading of winter wheat to the date of appearance of primary uredia at Lincoln, Nebr., 1921 to 1930*

| Year | Date of heading | Date of first uredia | Days from heading to first uredia | Length of the fruiting period | Days from first uredia to ripening |
|---|---|---|---|---|---|
| | | | *Number* | *Days* | *Number* |
| 1921 | May 26 | June 8 | 13 | 25 | 12 |
| 1922 | May 28 | May 30 | 2 | 27 | 25 |
| 1923 | June 6 | June 7 | 1 | 30 | 29 |
| 1924 | June 3 | June 20 | 17 | 33 | 16 |
| 1925 | May 29 | June 12 | 14 | 25 | 11 |
| 1926 | May 28 | June 8 | 11 | 27 | 16 |
| 1927 | June 7 | do | 1 | 29 | 28 |
| 1928 | May 29 | June 18 | 20 | 35 | 15 |
| 1929 | June 3 | June 9 | 6 | 30 | 24 |
| 1930 | May 27 | do | 13 | 34 | 21 |

## WEATHER CONDITIONS FAVORING THE DEVELOPMENT OF SECONDARY AND SUCCEEDING GENERATIONS OF UREDIOSPORES

It has been shown previously that the factors responsible for the development of secondary and succeeding infections are the length of the period from the appearance of primary uredia to ripening of the grain and optimum weather conditions favoring infection and rapid development of urediosporic generations.[8] It has been pointed out that in five years (1921, 1924, 1925, 1926, and 1928) this period averaged two weeks and was sufficiently short to produce not more than one or possibly two generations of urediospores under optimum weather conditions. In Table 6 the weather conditions prevailing during this interval are listed. Temperatures above the optimum for infection occurred in 1921 and 1925. In the other three years while temperatures slightly below the optimum prevailed, they were more favorable for rust development, although the period from initial infection to mature urediospores would be somewhat longer. On the other hand, precipitation necessary for infection was sufficient in all the five years except 1921, so that in the main the absence of rust development was due to the shortness of the period rather than to any specific environmental factor.

[8] Occasionally areas of heavier infection farther south abet in producing local rust epidemics. After several days of high southerly winds, followed by rains, a heavy rust infection sometimes develops in areas where primary uredia are few and widely scattered.

TABLE 6.—*Weather conditions prevailing for secondary and succeeding infections from the appearance of primary uredia to ripening of winter wheat at Lincoln, Nebr., 1921 to 1930*

| Year | Date of first uredia | Date of ripening | Days in period | Mean temperature for period | Amount of precipitation for period | Rainy days |
|---|---|---|---|---|---|---|
| | | | *Number* | *°F.* | *Inches* | *Number* |
| 1921 | June 8 | June 20 | 12 | 78.8 | 0.46 | 3 |
| 1922 | May 30 | June 24 | 25 | 74.9 | 1.20 | 7 |
| 1923 | June 7 | July 6 | 29 | 72.8 | .79 | 8 |
| 1924 | June 20 | ...do.... | 16 | 68.4 | 1.93 | 5 |
| 1925 | June 12 | June 23 | 11 | 75.9 | 4.74 | 6 |
| 1926 | June 8 | June 24 | 16 | 70.6 | 2.12 | 6 |
| 1927 | ...do.... | July 6 | 28 | 71.8 | 3.08 | 7 |
| 1928 | June 18 | July 3 | 15 | 70.3 | 1.92 | 8 |
| 1929 | June 9 | ...do.... | 24 | 74.1 | .44 | 6 |
| 1930 | ...do.... | June 30 | 21 | 73.2 | 2.60 | 5 |

During 1922, 1923, 1927, 1929, and 1930 the period from primary uredia to ripening of the grain was three to four weeks and hence was sufficiently long for several urediosporic generations to develop. In 1922, 1929, and 1930 relatively little secondary rust developed, and only a trace could be found in the wheat fields because of hot, dry weather. The year 1929 was deficient in rainfall for the entire period, whereas in 1922 most of the rainfall occurred after the wheat was maturing. In 1930 about one-half of the total rainfall for the period occurred in one day, five days after primary uredia made their appearance. Temperatures were more favorable for optimum infection in 1923 and 1927. Most of the rain fell during two days in 1927, about the middle of June, so that there was no moisture for further development of rust later in the period. In 1923, although the precipitation was light, it was scattered over a number of days. A trace of rain was also recorded for eight days in this period. Had there been more rainfall during this period in 1923 at Lincoln, much more rust would have occurred, and an epidemic as severe as that which took place in western Nebraska would have developed. During the 10 years there was no estimable loss from rust at Lincoln. The reasons vary with the years. In 5 of the 10 years the interval between the appearance of primary uredia and the ripening of the wheat was too short, and in 3 years this interval was long (more than three weeks), but hot, dry weather prevailed. In 1927 the development of uredia was inhibited by uneven rainfall over the latter part of the interval. In 1923 the amount of precipitation at any one time during the interval was too slight. In other words, rust did not assume epidemic proportions at Lincoln in any of the 10 years under discussion, although more rust occurred in 1922, 1923, and 1927 due in part to a greater amount of initial inoculum than in the other years under discussion. It is quite apparent that the limiting factor in the development of secondary and succeeding generations of urediospores, considering only the environmental factors, is the even distribution of an above normal precipitation.

## RELATION OF ENVIRONMENTAL FACTORS TO THE DEVELOPMENT OF STEM RUST IN NEBRASKA

Up to this point the discussion of the conditions favoring rust development has centered about the data collected at Lincoln, which can not be considered typical of the whole State. In order that the size of Nebraska can be visualized, the following statement can be given: If the State were pivoted at the southeast corner and moved in a 90° angle to the south, the western part would lie in Texas; and when pivoted 90° to the north, it would lie in Minnesota. The altitude increases at the rate of 1 foot for every 7 miles westward across the State, so that, with the exception of precipitation, conditions in the spring-wheat area in northwest Nebraska are somewhat similar to those in the spring-wheat area in Minnesota and the Dakotas.

For comparison, two points were chosen as representative of central and western Nebraska, namely, North Platte and Scottsbluff. At the North Platte substation, Turkey Red winter wheat heads, on the average, about 4 days later, ripens about 7 days later, and has a fruiting period 3 days longer than at Lincoln. The earliest date of heading was May 26 (1925) and the latest June 12 (1929), a range of 17 days. The earliest date of ripening was June 29 (1922 and 1925), and the latest July 18 (1929), a range of 19 days. The length of the fruiting period ranged from 26 days (1927) to 44 days (1928), a difference of 18 days, the average being 33 days for the 10-year period.[9]

On the average, primary uredia appear at North Platte about 10 days later than at Lincoln, the earliest date being June 12 and the latest June 28, a difference of 16 days. The average date of the appearance of primary uredia is June 20. On the whole, the range is not so great as at Lincoln. The number of days from heading to the appearance of primary uredia ranged from 6 to 29, and from primary uredia to ripening from 8 to 30. At North Platte, conditions vary as much as if not more than at Lincoln.

Unfortunately, no data on the length of the fruiting period of winter wheat are available at the substation near Scottsbluff. However, there would be less variation between Scottsbluff and North Platte than between North Platte and Lincoln. During seven years' observations, primary uredia appeared on the average of only two days later than at North Platte, the earliest date being June 17 and the latest June 28. In Table 7, the departures from normal at Lincoln, North Platte, Scottsbluff, and of the whole State, of mean monthly temperatures, precipitation, and rainy days for the months of May, June, and July are given.

It has previously been mentioned that a rust epidemic occurred in Nebraska in 1923, and that it was especially severe in the western half of the State. At North Platte temperatures were above normal during June and July, while precipitation was above normal for May, June, and July. Similar conditions prevailed at Scottsbluff, with even greater departures from the normal. Thus, while conditions were favorable for rust development in eastern Nebraska for only a short period after a large number of primary uredia appeared, conditions were much more favorable in central Nebraska and still more so in western Nebraska, especially in the spring-wheat section.

[9] Data supplied by L. L. Zook, associate agronomist, Division of Dry Land Agriculture, U. S. Department of Agriculture, North Platte, Nebr.

TABLE 7.—*Departures from normal of the mean temperatures, precipitation, and number of rainy days from May to July at Lincoln, North Platte, and Scottsbluff, Nebr., and for Nebraska, 1921–1930*

MAY

| Year | Lincoln | | | North Platte | | | Scottsbluff | | | Nebraska | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean temperature | Precipitation | Rainy days | Mean temperature | Precipitation | Rainy days | Mean temperature | Precipitation | Rainy days | Mean temperature | Precipitation | Rainy days |
| | ° *F.* | *Inches* | *Number* | ° *F.* | *Inches* | *Number* | ° *F.* | *Inches* | *Number* | ° *F.* | *Inches* | *Number* |
| 1921 | +3.6 | −0.98 | −3 | +2.3 | −0.89 | +1 | +0.3 | −0.78 | 0 | +1.6 | +0.09 | 0 |
| 1922 | +2.1 | −.86 | +3 | +.8 | −.25 | −2 | +1.6 | +.75 | −1 | +1.2 | −.76 | −1 |
| 1923 | −1.5 | −.77 | +1 | −1.5 | +1.30 | −1 | +.8 | +.46 | −1 | −1.6 | +1.46 | +2 |
| 1924 | −6.0 | −1.88 | 0 | −5.5 | −.52 | 0 | −3.5 | −.83 | +2 | −5.5 | −1.63 | −2 |
| 1925 | −1.1 | −2.83 | −3 | +.7 | −.86 | −4 | +3.9 | −.27 | +1 | −.4 | −1.47 | −3 |
| 1926 | +4.5 | −1.13 | +1 | +5.9 | −1.87 | +1 | +4.5 | −.61 | +3 | +5.0 | −.75 | +1 |
| 1927 | −.5 | −2.51 | −2 | +2.7 | −1.38 | 0 | +1.2 | −.20 | +2 | −.1 | −1.05 | −1 |
| 1928 | +2.9 | −1.33 | −4 | +3.8 | +.84 | +2 | +5.4 | −.42 | −5 | +3.1 | −.04 | 0 |
| 1929 | −1.7 | −.80 | −2 | −1.2 | −.20 | −1 | −.2 | −1.59 | −1 | −1.7 | −.80 | −2 |
| 1930 | −.3 | −.98 | +1 | −1.9 | +3.32 | +2 | −1.2 | +1.25 | +4 | −1.7 | +1.57 | +1 |
| Normal | 61.7 | 4.08 | 11 | 58.7 | 2.78 | 11 | 55.8 | 2.79 | 10 | 59.1 | 3.53 | 9 |

JUNE

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1921 | +5.0 | +0.11 | −2 | +4.3 | −1.83 | −2 | +3.5 | −1.07 | +2 | +4.0 | −1.43 | −2 |
| 1922 | +4.0 | −1.95 | +2 | +5.3 | −2.35 | −5 | +4.0 | −.64 | −1 | +3.2 | −1.37 | −3 |
| 1923 | +.7 | +.93 | +1 | +1.7 | +.93 | 0 | +.3 | +1.30 | +2 | +.3 | +.92 | +1 |
| 1924 | −2.3 | .00 | +6 | −.1 | −1.32 | −1 | +.9 | −1.88 | −3 | −1.9 | +.49 | +2 |
| 1925 | +2.8 | +2.32 | +2 | +4.2 | −1.76 | −2 | +1.0 | +1.53 | +1 | +1.9 | +.68 | +1 |
| 1926 | −.5 | −1.74 | −2 | +.5 | +.58 | +4 | −.2 | +1.11 | +2 | −.7 | −1.03 | −1 |
| 1927 | −1.8 | −.89 | −1 | −1.1 | +1.29 | +1 | −1.6 | +1.42 | +5 | −2.2 | −.12 | +1 |
| 1928 | −4.6 | −.35 | +6 | −4.7 | +1.42 | +9 | −6.0 | +1.16 | +6 | −6.0 | +.85 | +5 |
| 1929 | −1.0 | −2.46 | −2 | +1.1 | −1.98 | 0 | −.3 | +.06 | −2 | −1.4 | −.47 | −1 |
| 1930 | −.2 | −1.37 | −3 | +.9 | +.84 | +6 | +1.6 | −1.26 | −2 | −.8 | −.56 | −2 |
| Normal | 71.4 | 4.32 | 11 | 67.5 | 3.22 | 10 | 66.4 | 2.54 | 9 | 69.0 | 3.78 | 9 |

JULY

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1921 | +2.3 | +0.90 | +2 | +3.9 | −1.94 | −3 | +2.5 | −1.05 | −2 | +1.7 | +0.74 | +1 |
| 1922 | −2.7 | +2.38 | +6 | −.2 | +2.17 | +3 | −1.3 | +.68 | +1 | −2.1 | +1.20 | +3 |
| 1923 | +2.3 | −1.97 | −3 | +3.0 | −.89 | −2 | +2.2 | +1.14 | +2 | +1.8 | −.51 | 0 |
| 1924 | −3.6 | +.81 | +2 | −.3 | −1.74 | −3 | −.7 | −1.53 | 0 | −3.0 | +.41 | +1 |
| 1925 | +1.7 | −1.78 | −1 | +3.3 | −1.85 | −3 | +1.0 | −.18 | 0 | +.8 | −1.18 | 0 |
| 1926 | +2.1 | −2.01 | +1 | +2.9 | −.06 | +3 | −.1 | −.09 | 0 | +1.0 | −.59 | +1 |
| 1927 | −.1 | −1.97 | −1 | +1.0 | −2.31 | 0 | −1.7 | −.73 | 0 | −1.3 | −1.40 | −1 |
| 1928 | +.5 | +1.87 | +4 | +.5 | +2.30 | +5 | −.3 | +1.41 | +5 | −.7 | +.65 | +2 |
| 1929 | +.9 | +1.47 | 0 | +4.7 | −.68 | +2 | +3.0 | −.23 | −3 | +1.6 | −.17 | 0 |
| 1930 | +6.3 | −2.79 | −3 | +5.2 | −2.14 | −3 | +3.3 | −1.55 | −3 | +4.1 | −1.84 | −3 |
| Normal | 76.5 | 3.85 | 9 | 72.9 | 2.74 | 9 | 72.5 | 2.02 | 7 | 74.7 | 3.34 | 7 |

The year 1927 was potentially an epidemic year, since a large amount of initial inoculum reached Nebraska, and large numbers of primary uredia developed under favorable conditions. The lack of sufficient rainfall in July inhibited the development of secondary and succeeding generations of uredia over the whole State. In the remaining years, as at Lincoln, it was found that either mean temperatures were above normal, which in combination with a deficiency of rainfall (1921, 1922, 1925, 1926, 1929, 1930) inhibited the development of rust, or in occasional years an excess of precipitation (1928) was at hand, but mean temperatures were too low, or both temperatures and moisture were below normal (1924). In fact the right combination of factors favoring rust development in the western half of Nebraska is less likely to occur since mean temperatures are usually lower and the amount of precipitation is decidedly less than in eastern Nebraska. This can be seen by a comparision of normal temperatures and precipitation at the points listed in Table 7. Rust epidemics in central and

western Nebraska are possible, other factors being favorable, only when temperatures are above normal for June and July and an even distribution of rainfall well above normal prevails for these months.

## DISCUSSION

In Table 8 is a summary of some of the factors that have influenced the development of stem rust in Nebraska over a 10-year period. Weather plays a dominant rôle, since many other factors are dependent on it, at least in the different periods. The weather largely determines the time of heading and of ripening, and the length of the fruiting period of winter wheat. These are also important factors in the development of rust, once initial infection takes place. Each factor contributing to optimum rust development is influenced by a definite set of weather conditions, which are not necessarily the same for any other factor during the same or different seasons.

It has been pointed out that not only does initial inoculum appear at different times in June, in Nebraska, but the number and distribution of primary uredia vary from year to year. This variation is influenced (1) by the number and distribution of overwintering urediospores in Texas, (2) by the weather conditions affecting the increasing of a large amount of inoculum, which may progressively infect wheat to the northward, (3) by the receptiveness of wheat to infection when wind-blown urediospores reach Nebraska, and (4) by the prevailing environmental factors favoring the infection of the host. In Nebraska, no primary uredia have been found on winter-wheat plants, in the spring, prior to the late-boot or heading stage. While wind-blown urediospores may reach the State before the plant reaches this stage of development, infection does not take place because temperatures too low for infection usually prevail at this time.

In 1922, for example, a large amount of initial inoculum reached Nebraska early. Many primary uredia were found at Lincoln on May 30, at the time wheat was heading. A potential rust epidemic was in the making.

TABLE 8.—*Summary of factors influencing the development of stem rust, during a 10-year period, at Lincoln, Nebr., 1921–1930*

| Year | Time of heading | | | Time of first uredia | | | Time of ripening | | | Length of fruiting period | | | Interval from heading to first uredia | | | Interval from first uredia to ripening | | | Weather conditions from first uredia to ripening | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | | Mean temperature | | | Amount of precipitation | | | Number of rainy days | | |
| | Early | Intermediate | Late | Early | Intermediate | Late | Early | Intermediate | Late | Short | Intermediate | Long | Short | Intermediate | Long | Short | Intermediate | Long | High | Intermediate | Low | High | Intermediate | Low | High | Intermediate | Low |
| 1921 | X | | | | X | | | X | | X | | | | | X | X | | | X | | | | | X | | | X |
| 1922 | X | | | | X | | | X | | X | | | X | | | | | | X | | | | | X | | | |
| 1923 | | | X | | X | | | | X | | X | | X | | | | | X | | X | | | X | | X | | |
| 1924 | | X | | | | X | | | X | | | X | | | X | | X | | | | X | | | X | | | X |
| 1925 | X | | | X | | | | X | | X | | | | | X | | X | | X | | | X | | | | | X |
| 1926 | X | | | | X | | | X | | X | | | | | X | | X | | | | X | | | X | | | X |
| 1927 | | | X | | X | | | | X | | X | | X | | | | | X | | X | | | X | | | | |
| 1928 | X | | | | | X | | | X | | | X | | | X | | X | | | | X | | | X | | X | |
| 1929 | | X | | | X | | | X | | | X | | | X | | | | X | X | | | | | | | X | |
| 1930 | X | | | | X | | X | | | | | X | | | X | | X | | X | | | | X | | | X | |

Shortly thereafter, hot, dry weather accompanied by hot winds set in, which not only hastened the maturity of the wheat but also inhibited the rapid development of rust. Consequently the losses from rust during 1922 were negligible. Somewhat comparable conditions occurred in 1923 and 1927. In both years normal temperatures prevailed for optimum infection after primary uredia were found. Moisture, however, was deficient in eastern Nebraska in 1923, but in western Nebraska where temperatures and rainfall were above normal a severe rust epidemic occurred. In 1927 a deficiency in rainfall during late June and early July inhibited the further development of stem rust over the whole State.

On the other hand, only a small amount of initial inoculum reached Nebraska in 1924 and 1928. Primary uredia were not found until long after the plants were headed, and although the wheat ripened late, mean temperatures were too low for the rapid development of many generations of urediospores, so that only a trace of wheat stem rust occurred in Nebraska in these years. Thus in 1922 an epidemic was averted after a large amount of inoculum reached the State during the hot, dry weather, whereas in 1924 and 1928 the small amount of initial inoculum arrived too late to cause an epidemic. In general, it may be stated that temperature is the limiting factor for primary infection, whereas moisture is usually the limiting factor for secondary and succeeding infections in Nebraska.

A stem-rust epidemic is possible only with the following combination of factors: (1) A large amount of initial inoculum reaching Nebraska at a time when environmental factors favor maximum infection; (2) wheat entering the heading stage the first week in June or thereafter with primary uredia appearing at about this same time; and (3) an extended fruiting period during which temperature and moisture favorable for the development of many urediosporic generations prevail.

## SUMMARY

A study of the time and length of the fruiting period of wheat in a 28-year period shows that stem-rust epidemics did not occur in eastern Nebraska when winter wheat headed prior to, or during the first week in June and ripened before the first of July. The short fruiting period prevented the development of many urediosporic generations, and the environmental factors conducive to early maturity of winter wheat usually inhibited the rapid development of stem rust. These years may be classed as rust escaping. Rust epidemics occurred during occasional years within this period but were not correlated in all instances with an extended fruiting period because during other years with the same length of the fruiting period little or no rust developed.

An intensive study, over a 10-year period, of a number of factors influencing the development of stem rust leads to the conclusion that a rust epidemic is possible in the winter-wheat area of Nebraska only when a certain combination of factors is present in sequence. These factors are: (1) A large amount of initial inoculum reaching Nebraska when conditions favor maximum infection and the subsequent production of large numbers of primary uredia over a wide area; (2) winter wheat entering the heading stage the first week of June or thereafter, with primary uredia appearing at about the same time;

and (3) an extended fruiting period, during which optimum temperature, an evenly distributed precipitation above the normal, and other favorable conditions are at hand for the rapid development of urediosporic generations.

Apparently low temperatures are the major limiting factor in the development of primary infection and subsequent development of uredia, whereas the lack of an even distribution of sufficient precipitation is the major inhibiting factor in the development of subsequent urediosporic generations during most years.

Fortunately the sequence of factors necessary for stem-rust epidemics are fulfilled only in occasional years in the winter-wheat area of Nebraska. They may occur, however, in two successive years as in 1919 and 1920, or at intervals of as long as 12 years as in 1904 and 1916, with none between.

# INHERITANCE OF DWARFING IN WHEAT [1]

By D. C. TINGEY [2]

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## INTRODUCTION

Wheat crosses are made primarily to obtain new varieties of greater economic value. Frequently, however, in the process of breeding for new varieties it is possible to obtain valuable genetic data at little extra cost. This secondary phase of plant breeding, that of furnishing a genetic interpretation for the inheritance of a character, forms the basis of the present discussion.

Certain wheat crosses, involving normally tall varieties, will give rise to dwarf plants in the progeny. This peculiar characteristic is not common to wheat alone, but, according to Waldron (*12*),[3] has been reported in the breeding of other crop plants such as maize, barley, oats, peas, beans, squash, and tomatoes. However, not all dwarfs are alike; apparently there are different types even within the same species.

## REVIEW OF LITERATURE

The dwarf character in wheat usually does not appear until the $F_2$ generation, although some workers have observed dwarfs in the $F_1$ (*2*, *7*, *9*).

The presence of dwarfs in the $F_2$ and later generations has usually been explained on the basis of a 2-factor difference—one, a dominant dwarfing factor, the other an inhibiting factor. Clark and Hooker (*1*) in a Marquis × Hard Federation cross, Stephens (*10*) in a Federation × Master cross, and Stewart and Tingey (*11*) in a Federation × Marquis cross, explained their results on the 2-factor basis. Similar explanations were given by Clark and Quisenberry (*2*), Hayes and Aamodt (*6*), and Goulden (*5*) for results secured in crosses involving Marquis and Kota wheats. However, from a cross involving the same varieties (Marquis and Kota), Waldron (*12*) reported irregular breeding behavior in respect to the dwarf and tall plants, the ratios obtained in $F_2$ being 3:1, 13:3, 55:9, 15:1, and 63:1. These results he explained on the basis of a 3-factor difference—one factor the normal, *N*, and the other two complementary dwarf factors, *A* and *D*, both of which are inhibited in expression in the presence of *N*. Since this explanation does not account for all the ratios obtained, Waldron further concluded that the genes concerned are in a labile condition, often changing from dwarf to normal, and vice versa. In a Chul × Marquis cross, Goulden (*5*) found that two factors were involved. He obtained ratios in the $F_2$ which were difficult to explain on this

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[2] The writer expresses his appreciation to Mrs. Blanche C. Pittman for reading and correcting the manuscript and to Margaret Richards for calculating the data presented in the tables.

[3] Reference is made by number (italic) to Literature Cited, p. 94.

basis, but cytological studies gave evidence that lagging chromosomes were present in most of the cells undergoing reduction division. This, he believed, would account for the irregular breeding behavior in the $F_3$ generation.

From a study of the proportion of dwarfs in the $F_2$ generation of two crosses, Quality × Jenkin and Jenkin × Marquis, as well as of two back crosses (Quality × Jenkin) × Quality and (Jenkin × Marquis) × Marquis, Florell (*4*) concluded that there was a 3-factor difference between normal and dwarf. Neethling, according to Matsuura (*8*), reported that in tall × tall crosses he obtained in the $F_2$ generation 8 dwarfs in a total of 31 plants, or what was considered a 1:3 ratio. However, so few individuals are unreliable as a measure of the true ratio. Furthermore, these same data, if any reliance can be placed on results obtained with so few plants, are within the limits of the error, when considered on a 13:3 basis. If the study had been continued into the $F_3$ generation, the question as to whether the true ratio is 3:1 or 13:3 would have been answered.

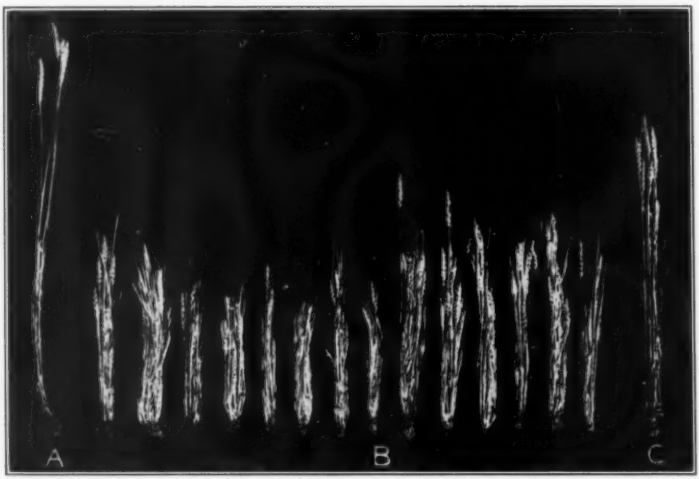


FIGURE 1.—Homozygous dwarf plants (B) in comparison with the two parents, Dicklow (A) and Hard Federation (C)

## EXPERIMENTAL METHODS AND MATERIAL

Crosses were made and carried through the different generations in the usual way. Pollen from the anthers of a single spike was used to pollinate the stigmas of a single spike. All seedings were made in the field. The genetic composition of the $F_2$ generation was determined by the breeding behavior of the $F_3$. In a number of crosses height data were taken on the $F_2$ plants, and a biometrical study was made of these data. The $F_3$ generation usually consisted of 200 or more rows except in some of the tall × dwarf crosses, where this number was not available. Each $F_3$ row was seeded from a single $F_2$ plant. The number of $F_3$ rows is shown in the various goodness-of-fit tables. The $F_3$ rows were usually 12 feet long and the kernels were spaced from 2 to 3 inches apart. This gave from 25 to 70 mature plants to the row.

In studying the goodness of fit, the $\chi^2$ test as given by Fisher (*3*) was used.

The dwarf plants referred to in this paper were somewhat variable in height, leafiness, and time of maturity. In these respects they were similar to tall varieties. Some dwarfs were short, grasslike clumps with few culms, which matured little, if any, seed; others were short but not so grassy and matured considerable seed when grown under favorable conditions; still others were rather grassy

FIGURE 2.—Relative length of the lower second or third internode in tall (A) and dwarf (B) plants

clumps with one or two culms nearly as long as those of the tall varieties. The last-mentioned type is usually heterozygous. In Figure 1 are shown some dwarfs from a homozygous row in comparison with the tall parents.

The dwarfing characteristic is the result of a shortening of the second or third internode near the base of the plant; sometimes both internodes are so shortened that the nodes appear to be almost together. (Fig. 2.) The upper internodes are somewhat shorter in dwarfs than in tall plants, but less markedly so than the lower.

## EXPERIMENTAL DATA

The data reported were obtained from a study of 92 different crosses involving some 50 or more strains and varieties of wheat. Detailed genetic studies, however, have been made of only a comparatively few of these 92 crosses. A number of varieties have been intercrossed in such a way as to provide an additional test of the breeding behavior of the character under consideration. The genetic composition of strains and varieties has been studied in three different ways: (1) By the usual method of studying the breeding behavior in the $F_2$ and $F_3$ generations involving tall × tall crosses; (2) by studying the breeding behavior in the $F_2$ and $F_3$ generations with tall × dwarf crosses; and (3) by intercrossing varieties in different combinations.

### TALL × TALL CROSSES

Of the crosses in which both parents were tall, 36 gave dwarfs in $F_2$. Eleven of these were studied. In 4 of the 11 the genetic composition of the $F_2$ dwarfs was determined by the $F_3$ breeding behavior, but in the remaining 7 only the tall $F_2$ plants were seeded for study of the $F_3$. These 7 crosses involved winter wheats. It was difficult to secure seed from dwarf plants grown under dry-land conditions, and for this reason it was not possible to continue the study of the dwarfs into the $F_3$ generation.

TABLE 1.—*Classification of $F_2$ plants into tall and dwarf and the calculated $\chi^2$ test for goodness of fit based on a 13:3 ratio*

| Cross No. | Parents | Class | Observed | Calculated | $\chi^2$ | P |
|---|---|---|---|---|---|---|
| 23a | Dicklow × No. 4 Sevier No. 121 | Tall | 407 | 400.6 | 0.4535 | 0.50–0.70 |
| | | Dwarf | 86 | 92.4 | | |
| 35c | Dicklow No. 3 × F–22 (Dicklow×Sevier) | Tall | 212 | 208.8 | .2614 | .50–.70 |
| | | Dwarf | 45 | 48.2 | | |
| 39a | Dicklow No. 3 × Hard Federation | Tall | 256 | 252.7 | .2299 | .50–.70 |
| | | Dwarf | 55 | 58.3 | | |
| 47e | Marquis × Hard Federation | Tall | 216 | 216.9 | .0199 | .80–.90 |
| | | Dwarf | 51 | 50.1 | | |

### SPRING-WHEAT CROSSES

#### $F_2$ DATA

Seed from the dwarfs in the spring-wheat crosses grown under irrigation generally germinated well if planted reasonably early. Height measurements were taken on the $F_2$ plants in the four spring-wheat crosses involving tall parents, and the plants were classified as tall and dwarf. The results of this classification are shown in Table 1. In Table 1 the data are fitted to a 13:3 ratio. The assumption is that the contrasted characters, tall and dwarf, differ by two genetic factors—a dominant dwarfing factor (*DD*) and an inhibiting factor (*II*). When two varieties are crossed and dwarfs occur in the $F_2$ generation, it is thought that one parent must carry both dominant factors, *DDII*, and the other must carry both recessive factors, *ddii*. When these two types are crossed and the $F_1$ is selfed the theoretical genetic composition and breeding relationships shown in Table 2 should be obtained. This table indicates that theoretically at least the $F_2$ phenotypic ratio should be 13 tall:3 dwarf. If this be true, a similar segregating ratio would be expected when certain tall varieties are crossed. (Fig. 3.)

FIGURE 3.—Results in $F_1$ and $F_2$ from a tall (*ddii*)×tall (*DDII*) cross: A, Dicklow parent (*ddii*); B, Hard Federation parent (*DDII*); C, $F_1$ tall (*DdIi*); D, $F_2$ dwarf, 3; E, $F_2$, tall, 13

TABLE 2.—*Theoretical breeding behavior based on a 2-factor difference, one a dominant dwarfing factor (DD) and the other an inhibiting factor (II)*

Zygotes: *DDII* (tall) × *ddii* (tall); $F_1$, *DdIi* (tall)]

| $F_2$ phenotypic ratio | Proportion of $F_2$ genotypes | Genotype | $F_3$ breeding behavior | $F_3$ breeding ratio |
|---|---|---|---|---|
| 13 tall | 1 | DDII | Tall | 7 |
| | 2 | DdII | do | |
| | 1 | ddII | do | |
| | 2 | ddIi | do | |
| | 1 | ddii | do | |
| | 4 | DdIi | 13 tall : 3 dwarf | 4 |
| 3 dwarf | 2 | DDIi | 3 tall : 1 dwarf | 2 |
| | 2 | Ddii | 1 tall : 3 dwarf | 2 |
| | 1 | DDii | Dwarf | 1 |
| Total | 16 | | | 16 |

The four crosses studied in $F_2$ approximate this proportion of tall and dwarf plants. (Table 1.) The $\chi^2$ test for goodness of fit shows the value of $P$ to be large enough in all cases for the proportion of tall and dwarf to be considered well within the range of the sampling error. If $P$ is between 0.1 and 0.9, there is no reason to suspect the hypothesis tested; and even if the value of $P$ is as low as 0.05, the discrepancy is not serious (*3*).

### $F_3$ DATA

Seed of both tall and dwarf $F_2$ plants from each of the crosses listed in Table 1 was sown. Theoretically, if the 2-factor difference (*DDII* and *ddii*) is the correct explanation for the inheritance of dwarfing, then the segregation in $F_3$ should be as shown in Table 2;

that is, the $F_3$ rows should give a ratio of 7:4:2:2:1. The 7 should all be tall; the 4 should segregate 13 tall:3 dwarf; 2 should segregate 3 tall:1 dwarf; 2 should segregate 1 tall:3 dwarf; and 1 should be true dwarf.

Ratios of 13:3 and 3:1 are so nearly alike that it would be difficult to know which was the true one without further tests, unless large numbers of individuals were available in each case. In the $F_3$ rows, however, the number of plants is usually rather small; therefore, an attempt to classify the rows segregating 13:3 and 3:1 would be of doubtful value. It may appear that the deviates in the two ratios would tend to overlap; however, since these two ratios occur in the proportion of 4:2, an error is introduced, for the overlapping deviates would be more in one direction than in the other. In view of these complications, no attempt has been made to separate these two classes in the $F_3$ population. When the two classes are combined, the ratio becomes 7:6:2:1 instead of 7:4:2:2:1. The goodness-of-fit tests are shown in Table 3. Here again the value of $P$ is in all instances sufficiently large to be well within the expected limits.

TABLE 3.—*Goodness of fit from $F_3$ breeding classes based on a 7:6:2:1 ratio obtained in various crosses*

| Cross No. | Parents | Observed | Calculated | $\chi^2$ | P |
|---|---|---|---|---|---|
| 23a | Dicklow No. 4 × Sevier 121 | 210 | 215.7 | 1.4723 | 0.60–0.70 |
| | | 197 | 184.9 | | |
| | | 56 | 61.6 | | |
| | | 30 | 30.8 | | |
| 35c | Dicklow No. 3 × F-22 (Sevier × Dicklow) | 107 | 108.5 | .5637 | .90–.95 |
| | | 98 | 93.0 | | |
| | | 29 | 31.0 | | |
| | | 14 | 15.5 | | |
| 39a | Dicklow × Hard Federation | 135 | 133.4 | .5252 | .90–.95 |
| | | 116 | 114.4 | | |
| | | 34 | 38.1 | | |
| | | 20 | 19.1 | | |
| 47e | Marquis × Hard Federation | 108 | 116.3 | 1.2461 | .70–.80 |
| | | 107 | 99.7 | | |
| | | 35 | 33.2 | | |
| | | 16 | 16.6 | | |

## WINTER-WHEAT CROSSES

Seven of the winter-wheat crosses segregated for tall and dwarf in the $F_2$ generation. The dwarf plants were not carried into the $F_3$ for reasons previously mentioned. Because of this it is possible to determine only the breeding behavior of the tall plants. The proportion of tall and dwarf plants in the $F_2$ generation in the winter-wheat crosses was extremely variable, owing possibly to the abnormal development which took place under dry-land conditions. Since the dwarf plants were usually short grassy clumps, it was difficult to determine in some cases whether the small dead plants were dwarfs or whether they were potentially tall plants that had been winter-killed. This uncertainty of classification in $F_2$ would introduce a serious error and thus make the goodness-of-fit test unreliable, whereas in $F_3$ it was much simpler to list true-breeding or segregating rows, since more plants were involved; consequently, the $F_3$ data should be reliable.

### $F_3$ DATA

On the basis of the data shown in Table 2, the tall $F_2$ plants should segregate in $F_3$ in the proportion of 7 (homozygous tall):6 (segregating). Fitted to this expected 7:6 ratio, the results from the seven crosses are shown in Table 4. The value of $P$ is 0.1 or more in all cases. From these and the preceding results it appears that the 2-factor theory explains the inheritance of dwarfing in these crosses. Consequently, the genetic composition of the tall parents which on crossing throws dwarfs in $F_2$ are *DDII* and *ddii*.

TABLE 4.—*Goodness of fit for two classes: (1) True breeding tall, (2) segregated tall and dwarf (13:3 and 3:1 classes) in $F_3$ generation based on a 7:6 ratio*

| Cross No. | Parents | Class | Observed | Calculated | $\chi^2$ | P |
|---|---|---|---|---|---|---|
| 67a | Ridit × Sevier 59 | Tall | 131 | 134.6 | 0.2086 | 0.50–0.70 |
| | | Segregating | 119 | 115.4 | | |
| Do | do | Tall | 157 | 159.9 | .1139 | .70–.80 |
| | | Segregating | 140 | 137.1 | | |
| 69a | Ridit × Utac | Tall | 126 | 129.2 | .1717 | .50–.70 |
| | | Segregating | 114 | 110.8 | | |
| Do | do | Tall | 207 | 212.7 | .3309 | .50–.70 |
| | | Segregating | 188 | 182.3 | | |
| 70a | Ridit × 14–85 (Federation × Sevier) | Tall | 136 | 134.6 | .0316 | .80–.90 |
| | | Segregating | 114 | 115.4 | | |
| 103a | Federation × Martin | Tall | 151 | 156.2 | .3752 | .50–.70 |
| | | Segregating | 139 | 133.8 | | |
| 107a | Federation × Hohenheimer | Tall | 117 | 120.6 | .2328 | .50–.70 |
| | | Segregating | 107 | 103.4 | | |

### TALL × DWARF CROSSES

Tall × dwarf crosses were made to determine more definitely whether or not the 2-factor theory is correct and also to ascertain which variety or varieties carry the dwarfing factor. These tall × dwarf crosses are listed in Table 5. Homozygous dwarfs were used as the dwarf parents. The tall parents were varieties that had been used in previous studies of tall × tall crosses.

TABLE 5.—*Varieties used in the tall × dwarf crosses and type of plants in the $F_1$*

| Cross No. | Female parent | Male parent | $F_1$ tall or dwarf |
|---|---|---|---|
| 60 | Dicklow No. 3 | Q 100–4 (dwarf) | Dwarf. |
| 61 | do | Q 114–14–3 (dwarf) | Do. |
| 62 | do | Q 100–1 (dwarf) | Do. |
| 63 | do | Q 114–13 (dwarf) | Do. |
| 64 | do | Q 136–17–1 (dwarf) | Do. |
| 65 | do | Q 100–2 (dwarf) | Do. |
| 96 | Federation | 39a–304 (dwarf) | Tall. |
| 97 | Sevier No. 59 | do | Do. |
| 98 | Hard Federation | do | Do. |
| 99 | Marquis | do | Dwarf. |

If the tall × tall crosses segregating for tall and dwarfs in $F_2$ are *DDII* and *ddii*, then the homozygous dwarf should be *DDii*. The genetic composition and theoretical breeding behavior of these *DDII* (tall) × *DDii* (dwarf) and *ddii* (tall) × *DDii* (dwarf) are shown in Table 6. It will be noted that both of the tall types differ from the dwarf in only one factor pair. Hence, crosses with dwarfs ought to give ratios of either 3 tall:1 dwarf (or 1:2:1) or 1 tall:3 dwarf

(or 1:2:1) in $F_2$ and a ratio of 1:2:1 in $F_3$. The $F_1$ generation will show the genetic composition of the tall varieties, since the genetic type *DDII* (tall) × *DDii* (dwarf) cross will give *DDIi*, which will be tall in $F_1$, whereas the genetic type *ddii* (tall) × *DDii* (dwarf) will give *Ddii*, which will be dwarf in $F_1$. (Figs. 4 and 5.)

TABLE 6.—*Genetic composition and theoretical breeding behavior of tall (DDII) × dwarf (DDii) and tall (ddii) × dwarf (DDii)*

| Parents | $F_1$ | $F_2$ phenotypic ratio | $F_3$ breeding behavior |
|---|---|---|---|
| Dwarf (*DDii*) × tall (*DDII*) | tall (*DDIi*) | 3 tall; 1 dwarf or (1:2:1) | 1 tall, 2 segregating, 1 dwarf. |
| Dwarf (*DDii*) × tall (*ddii*) | dwarf (*Ddii*) | 1 tall; 3 dwarf or (1:2:1) | 1 tall, 2 segregating, 1 dwarf. |

## $F_2$ DATA

The $F_2$ data secured from studies involving two genetic types of tall crossed with dwarf are given in Table 7. It will be observed that in all cases the goodness of fit as measured by $P$ is 0.10 or more. Figures 4 and 5 show the results of crossing each of the tall types *DDII* and *ddii* with dwarf.

TABLE 7.—*Goodness of fit for two $F_2$ phenotypes based on ratio of either 3 tall : 1 dwarf or 1 tall : 3 dwarf*

| Cross No. | Parents [a] | $F_1$ | $F_2$ class | Observed | Calculated | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|
| 60a | Dicklow × dwarf (q100–4) | Dwarf | Tall | 9 | 10.2 | 0.1880 | 0.50–0.70 |
| | | | Dwarf | 32 | 30.8 | | |
| 60e | do | do | Tall | 11 | 11.7 | .0558 | .80–.90 |
| | | | Dwarf | 36 | 35.3 | | |
| 60g | do | do | Tall | 15 | 13 | .4103 | .50–.70 |
| | | | Dwarf | 37 | 39 | | |
| 96a | Federation × dwarf (39a–304) | Tall | Tall | 193 | 192 | .0207 | .80–.90 |
| | | | Dwarf | 63 | 64 | | |
| 97a | Sevier 59 × dwarf (39a–304) | do | Tall | 197 | 193.5 | .2532 | .50–.70 |
| | | | Dwarf | 61 | 64.5 | | |
| 98 | Hard Federation × dwarf (39a–304) | do | Tall | 181 | 180 | .0723 | .70–.80 |
| | | | Dwarf | 59 | 60 | | |
| 99 | Marquis × dwarf (39–304) | Dwarf | Tall | 55 | 46.5 | 2.0717 | .10–.20 |
| | | | Dwarf | 131 | 139.5 | | |
| 63b | Dicklow × dwarf (q114–13) | do | [b] | | | | |

[a] Numbers in parentheses refer to dwarf number.

[b] Difficult to classify.

## $F_3$ DATA

Both tall and dwarf plants were seeded for a study of the $F_3$ progeny. The data obtained were fitted to the expected ratio of 1 tall:2 segregating:1 dwarf. (Table 8.) It is obvious that in all crosses, except 63b, Dicklow × Dwarf (q114–13) the observed ratios fit the expected when tested by $\chi^2$. The plants in the $F_2$ generation of this cross were difficult to classify. Dominance was apparently incomplete. The $F_2$ plants varied in length of culm from dwarf to tall. Some plants developed an unusually heavy vegetative growth. In this respect they resembled the dwarf type, yet these same plants had culms ranging in length from those characteristic of dwarfs to those characteristic of the tall varieties. Because of this difficulty, goodness-of-fit data for cross 63b in the $F_2$ generation were not included in Table 7.

FIGURE 4.—Results in $F_1$ and $F_2$ from a tall (*DDII*) × dwarf (*DDii*): A, Hard Federation parent (*DDII*); dwarf parent (*DDii*); C, tall $F_1$ (*DDIi*); D, $F_2$ dwarf, 1; E, $F_2$ tall, 3. Compare with Figure 5

FIGURE 5.—Results in the $F_1$ and $F_2$ from a tall (*ddii*) × dwarf (*DDii*) cross: A, Dicklow parent (*ddii*); B, dwarf parent (*DDii*); C, dwarf $F_1$ (*Ddii*); D, $F_2$ tall, 1; E, $F_2$ dwarf, 3. Compare with Figure 4

TABLE 8.—*Goodness of fit for three $F_3$ breeding classes based on a 1:2:1 ratio as obtained in various crosses involving tall and dwarf types*

| Cross No. | Parents [a] | Class | Observed | Calculated | $\chi^2$ | P |
|---|---|---|---|---|---|---|
| 60a | Dicklow × dwarf (q100–4) | Tall | 9 | 10.2 | 0.2161 | 0.80–0.90 |
| | | Segregating | 21 | 20.5 | | |
| | | Dwarf | 11 | 10.2 | | |
| 60e | do | Tall | 11 | 11.5 | .7391 | .50–.70 |
| | | Segregating | 21 | 23.0 | | |
| | | Dwarf | 14 | 11.5 | | |
| 60g | do | Tall | 15 | 13.0 | 1.1385 | .50–.70 |
| | | Segregating | 27 | 26.0 | | |
| | | Dwarf | 10 | 13.0 | | |
| 63b | Dicklow × dwarf (q114–13) | Tall | 32 | 31.5 | 16.0159 | .0001–.0003 |
| | | Segregating | 81 | 63.0 | | |
| | | Dwarf | 13 | 31.5 | | |
| 96 | Federation × dwarf (39a–304) | Tall | 65 | 64.0 | .0312 | .95–.98 |
| | | Segregating | 128 | 128.0 | | |
| | | Dwarf | 63 | 64.0 | | |
| 97 | Sevier 59 × dwarf (39a–304) | Tall | 74 | 66.5 | 2.2331 | .30–.50 |
| | | Segregating | 135 | 133.0 | | |
| | | Dwarf | 57 | 66.5 | | |
| 98 | Hard Federation × dwarf (39a–304) | Tall | 54 | 60.0 | 1.0250 | .50–.70 |
| | | Segregating | 127 | 120.0 | | |
| | | Dwarf | 59 | 60.0 | | |
| 99 | Marquis × dwarf (39a–304) | Tall | 52 | 45.7 | 2.4780 | .20–.30 |
| | | Segregating | 81 | 91.5 | | |
| | | Dwarf | 50 | 45.7 | | |

[a] Numbers in parentheses refer to dwarf numbers.

Some of these intermediate types of plants, when seeded in $F_3$, segregated for tall and dwarf; others bred true for tall; and still others bred true for dwarf, though the dwarfs were taller than usual. A few appeared to breed true for this intermediate characteristic. However, when plants were selected from these and seeded in $F_4$, some bred true for tall, others for dwarf, and still others segregated, thus showing that these intermediates were heterozygous. The true-breeding tall plants selected from these intermediate rows appeared shorter than the tall parent used in the cross. This shortening of the culm length might be traceable to Hard Federation, since this variety was one of the original parents used in the cross from which was selected the dwarf plant employed as a parent in cross 63b. There are, doubtless, factors other than the dwarfing factor which influence the length of culm, since there are varieties with different culm lengths which show no dwarfing characteristics; likewise, there are dwarfs with different culm lengths.

It is interesting to note from the goodness-of-fit studies in Table 8, that in cross 63b (Dicklow × dwarf), the number of true-breeding tall plants was almost exactly as expected. By grouping the other two classes (the segregating and the dwarf) into one, a good fit to a 3:1 ratio is obtained. The discrepancy in this cross is due to the fact that there are too many in the segregating group and too few in the true dwarf group on the basis of a 1:2:1 ratio. This discrepancy may be accounted for by assuming that some natural crossing took place in the field among the $F_2$ plants. This is a perfectly logical assumption, since it has been shown that in some wheats, under certain conditions, there may be a high percentage of crossing (7). Crossing occurs more often on abnormally than on normally developed spikes. Abnormally developed spikes are more or less common in dwarfs. It was on just such plants as these (dwarfs) that natural crossing is believed to have occurred. The Dicklow is known to be of the *ddii*

type. The $F_1$ plant was a dwarf and consequently should have given a ratio, in $F_2$, of 3 dwarfs:1 tall, or 1:2:1 if dominance was incomplete. Thus, all tall plants in $F_2$ should breed true for tallness in $F_3$. Two of the $F_2$ dwarfs should segregate in $F_3$ and the third should breed true. It so happened, however, that a larger proportion segregated. Obviously, it could be supposed that when the $F_2$ plants were in bloom pollen from tall plants fell on the lower dwarfs and caused fertilization. If pollen from a tall plant, *dd*, were to fertilize a dwarf, *DD*, a dwarf or intermediate plant in $F_2$ would result. Instead, therefore, of being *DD* (true dwarf), it would be *Dd*, and consequently would segregate in $F_3$, thus giving a proportionately higher percentage of segregates than would have occurred under normal self-fertilization. Part of this effect would be counterbalanced if heterozygous dwarfs, *Dd*, were pollinated by tall plants, *dd*, since the proportion of dwarf and tall would be reduced from 3 dwarf:1 tall to 1 dwarf:1 tall.

It would be very difficult for the pollen from the dwarfs to fertilize the tall plants. Moreover, the spikes on the tall plants develop more normally, thus excluding the possibility of any appreciable number of tall plants being fertilized by pollen from dwarf plants; consequently, this group would behave normally, thus accounting for the close approach to the expected ratio.

### NATURAL CROSSING BETWEEN TALL AND DWARF PLANTS

Since it was suspected that natural crossing occurred, and since this provided a logical explanation of the irregular breeding behavior of one of the tall × dwarf crosses, it appeared desirable to determine whether or not there was natural crossing from tall to dwarf plants. In one part of the grain nursery a series of rows was sown with seed from homozygous dwarfs and from the original tall parents of the cross from which the dwarfs had been selected. The dwarf strain in one of these rows had been grown for several years and had been used in a number of tall × dwarf crosses. Seed from this dwarf was sown about 4 feet from Hard Federation. Since Hard Federation is of the *DDII* type, it was possible to determine whether or not crossing occurred by simply making seedings from plants selected from the dwarf row. This was possible because of the fact that the composition of these dwarf plants is *DDii* and any crossing that might occur with a *DDII* (tall) plant would give rise to a plant of the composition *DDIi*; that is, a tall plant.

Twenty-five plants were chosen at random from the dwarf row, and seeds from each were sown the following year in single 12-foot rows. This gave about 40 mature plants to the row. A similar row was sown from 2-year-old seed of the kind sown to produce the dwarf row from which selections were made to test the amount of natural crossing with Hard Federation. Later in the season it was obvious that natural crossing had occurred, since 13 of the 25 rows, or 52 per cent, contained both tall and dwarf plants. (Table 9.) In these 25 rows the percentage of natural crossing ranged from 0 to 7.6, with an average of 1.8. The row planted from 2-year-old seed contained no tall plants.

Further evidence of natural crossing was found when a study was made of the inheritance of dwarfing in $F_3$ rows, for in some rows one or two tall plants were observed, whereas all were expected to be dwarfs.

TABLE 9.—*Natural crossing of tall (DDII) and dwarf (DDii) when grown 4 feet from each other in the field*

| Source of seed | Rows | Approximate plants to the row | Natural crossing | | |
|---|---|---|---|---|---|
| | | | Rows | Range in percentage of rows | Average percentage of total plants showing natural crossing |
| | *Number* | *Number* | *Per cent* | | |
| Dwarf, 39a-304, 1930 seed | 1 | 40 | 0 | 0 | 0 |
| Selected dwarfs from 39a-301, 1931 seed | 25 | 40 | 52 | 0-7.6 | 1.8 |

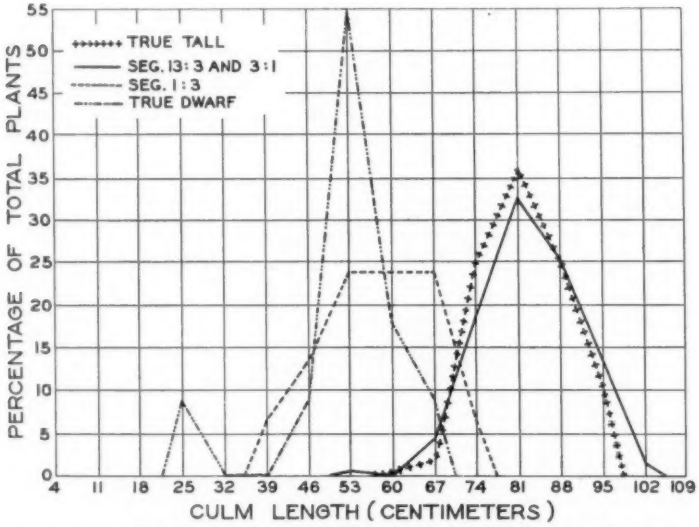


FIGURE 6.—Distribution of culm length of the $F_2$ plants for different breeding classes in $F_3$ in the cross Dicklow 3 × F-22 (Sevier × Dicklow). (For convenience a whole number was taken for the class center)

## BIOMETRICAL STUDY OF HEIGHT IN TALL AND DWARF $F_2$ PLANTS

Height measurements were taken on the longest culm of each $F_2$ plant in a number of crosses. These plants were later classified on the basis of the $F_3$ breeding behavior. Statistical constants were calculated for each class. (Table 10 and figs. 6 to 10.) In the crosses Dicklow 3 × Hard Federation and Marquis × Hard Federation, the range in dwarf plants in $F_2$ was distinctly outside that of the tall plants. (Table 10.) The range and the mean height for the segregating or heterozygous dwarfs were not materially different from those for the homozygous. There was considerable overlapping of tall and dwarf in the Dicklow 3 × F-22 (Sevier × Dicklow) cross. This overlapping of tall and dwarf was characteristic also of the Dicklow 3 × Hard Federation.

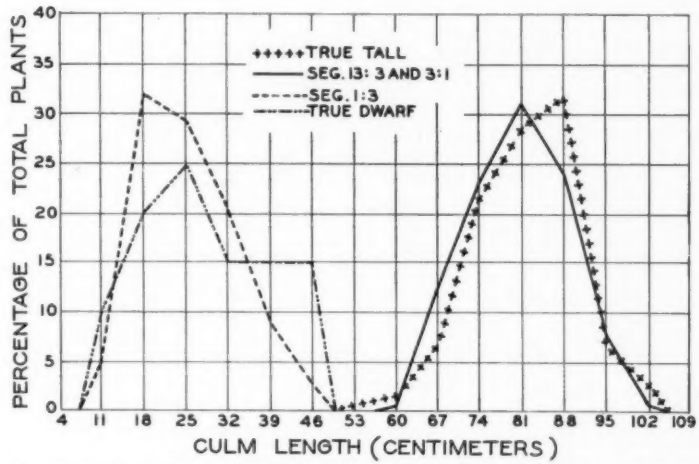
++++TRUE TALL
SEG. 13: 3 AND 3:1
SEG. 1:3
TRUE DWARF
PERCENTAGE OF TOTAL PLANTS
0 5 10 15 20 25 30 35 40
4 11 18 25 32 39 46 53 60 67 74 81 88 95 102 109
CULM LENGTH (CENTIMETERS)

FIGURE 7.—Distribution of culm length of the $F_2$ plants for different breeding classes in $F_3$ in the cross Dicklow 3 × Hard Federation. (For convenience a whole number was taken for the class center)

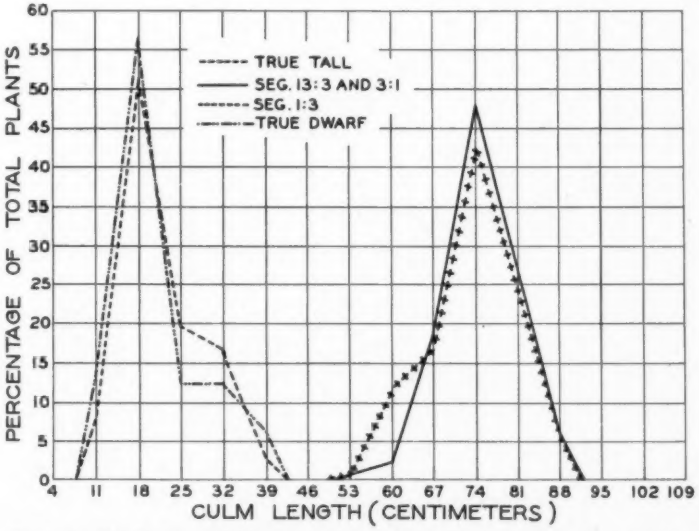
TRUE TALL
SEG. 13: 3 AND 3:1
SEG. 1:3
TRUE DWARF
PERCENTAGE OF TOTAL PLANTS
0 5 10 15 20 25 30 35 40 45 50 55 60
4 11 18 25 32 39 46 53 60 67 74 81 88 95 102 109
CULM LENGTH (CENTIMETERS)

FIGURE 8.—Distribution of culm length of the $F_2$ plants for different breeding classes in $F_3$ in the cross Marquis × Hard Federation. (For convenience a whole number was taken for the class center)

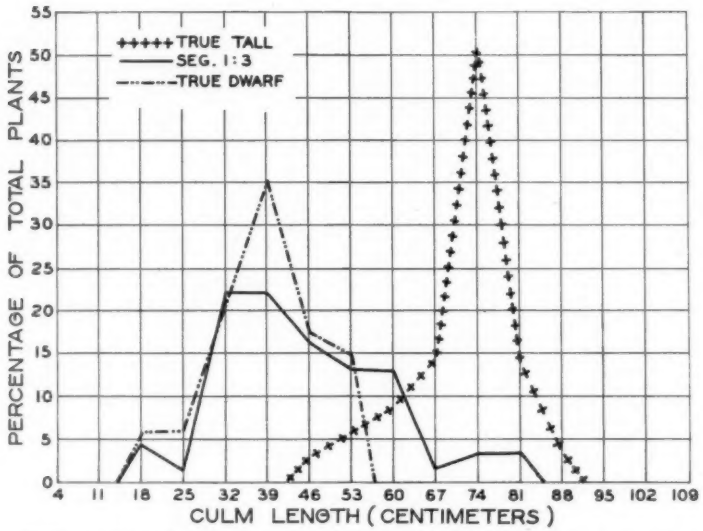
+++++TRUE TALL
SEG. 1:3
-·-·-TRUE DWARF
PERCENTAGE OF TOTAL PLANTS
0 5 10 15 20 25 30 35 40 45 50 55
4 11 18 25 32 39 46 53 60 67 74 81 88 95 102 109
CULM LENGTH (CENTIMETERS)

FIGURE 9.—Distribution of culm length of the $F_2$ plants for different breeding classes in $F_3$ in the cross Dicklow × Dwarf (q100-4). (For convenience a whole number was taken for the class center)

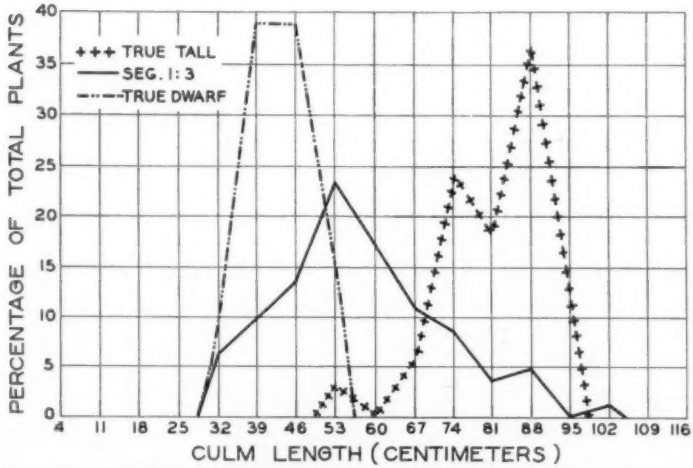
+++ TRUE TALL
SEG. 1:3
-·-·-TRUE DWARF
PERCENTAGE OF TOTAL PLANTS
0 5 10 15 20 25 30 35 40
4 11 18 25 32 39 46 53 60 67 74 81 88 95 102 109 116
CULM LENGTH (CENTIMETERS)

FIGURE 10.—Distribution of culm length of the $F_2$ plants for different breeding classes in $F_3$ in the cross Dicklow × Dwarf (q114-13). (For convenience a whole number was taken for the class center)

TABLE 10.—*Frequency distribution for the height of the $F_2$ plants for various breeding classes in $F_3$*

| Cross | Breeding class | Number in plant-height class (cm) indicated | | | | | | | | | | | | | Total | Mean | S. D. | C. V. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 10.5 | 17.5 | 24.5 | 31.5 | 38.5 | 45.5 | 52.5 | 59.5 | 66.5 | 73.5 | 80.5 | 87.5 | 94.5 | 101.5 | | | | |
| 35c | Dicklow 3×F–22 (Sevier×Dicklow): | | | | | | | | | | | | | | | | | | |
| | a, True tall | | | | | | | | 1 | 3 | 26 | 39 | 27 | 11 | | 107 | 81.4±0.47 | 7.3±0.34 | 8.9±0.41 |
| | b, Segregating 13:3, 3:1 | | | | | | | 1 | 0 | 5 | 19 | 31 | 26 | 14 | 2 | 98 | 82.4± .59 | 8.6± .41 | 10.4± .50 |
| | c, Segregating 1:3 | | | | | 2 | 4 | 7 | 7 | 7 | 2 | | | | | 29 | 57.1±1.14 | 9.0± .80 | 15.7±1.42 |
| | d, True dwarf | | | 1 | 0 | 0 | 1 | 6 | 2 | 1 | | | | | | 11 | 51.8±2.16 | 10.1±1.45 | 19.5±2.91 |
| 39a | Dicklow 3×Hard Federation: | | | | | | | | | | | | | | | | | | |
| | a, True tall | | | | | | | 1 | 2 | 9 | 28 | 39 | 43 | 9 | 4 | 135 | 81.4± .48 | 8.3± .34 | 10.1± .42 |
| | b, Segregating 13:3, 3:1 | | | | | | | | 1 | 14 | 27 | 36 | 28 | 9 | 1 | 116 | 80.1± .52 | 8.3± .36 | 10.3± .46 |
| | c, Segregating 1:3 | 2 | 11 | 10 | 7 | 3 | 1 | | | | | | | | | 34 | 24.7± .97 | 8.3± .68 | 33.7±3.78 |
| | d, True dwarf | 2 | 4 | 5 | 3 | 3 | 3 | | | | | | | | | 20 | 28.0±1.79 | 11.6±1.24 | 41.5±5.14 |
| 47e | Marquis ×Hard Federation: | | | | | | | | | | | | | | | | | | |
| | a, True tall | | | | | | | 1 | 12 | 18 | 46 | 25 | 6 | | | 108 | 73.0± .46 | 7.1± .32 | 9.8± .45 |
| | b, Segregating 13:3, 3:1 | | | | | | | 1 | 3 | 19 | 51 | 27 | 6 | | | 107 | 74.2± .42 | 6.4± .30 | 8.7± .40 |
| | c, Segregating 1:3 | 3 | 18 | 7 | 6 | 1 | | | | | | | | | | 35 | 21.3± .79 | 6.9± .55 | 32.2±2.85 |
| | d, True dwarf | 2 | 9 | 2 | 2 | 1 | | | | | | | | | | 16 | 20.6±1.34 | 7.7± .92 | 37.4±5.04 |
| 60 (a-e-g) | Dicklow×Dwarf (Q100–4): | | | | | | | | | | | | | | | | | | |
| | a, True tall | | | | | | 1 | 2 | 3 | 5 | 18 | 5 | 1 | | | 35 | 70.7±1.02 | 8.9± .71 | 12.6±1.03 |
| | b, Segregating 1:3 | | 3 | 1 | 15 | 15 | 11 | 9 | 9 | 1 | 2 | 2 | | | | 68 | 44.2±1.13 | 13.8± .80 | 31.2±1.99 |
| | c, True dwarf | | 2 | 2 | 7 | 12 | 6 | 5 | | | | | | | | 34 | 38.3±1.09 | 9.4± .77 | 24.5±2.12 |
| 63b | Dicklow×Dwarf (Q114–13): | | | | | | | | | | | | | | | | | | |
| | a, True tall | | | | | | | 1 | 0 | 2 | 8 | 6 | 12 | 4 | | 33 | 81.3±1.13 | 9.5± .79 | 11.7± .98 |
| | b, Segregating 1:3 | | | | 5 | 8 | 11 | 19 | 14 | 9 | 7 | 3 | 4 | 0 | 1 | 81 | 56.7±1.12 | 14.9± .79 | 26.2±1.49 |
| | c, True dwarf | | | | 1 | 5 | 5 | 2 | | | | | | | | 13 | 42.8±1.10 | 5.9± .78 | 13.7±1.86 |
| 69a | Ridit×Utac (Dicklow×Sevier): | | | | | | | | | | | | | | | | | | |
| | a, True tall | | | | | | | | 1 | 4 | 25 | 54 | 42 | | | 126 | 80.8± .36 | 5.9± .25 | 7.3± .31 |
| | b, Segregating 13:3, 3:1 | | | | | | | | | 2 | 30 | 59 | 20 | 3 | | 114 | 80.2± .35 | 5.5± .25 | 6.8± .31 |
| 70a | Ridit×14–85 (Federation×Sevier): | | | | | | | | | | | | | | | | | | |
| | a, True tall | | | | | | | | 5 | 41 | 64 | 25 | 1 | | | 136 | 72.2± .32 | 5.5± .23 | 7.7± .31 |
| | b, Segregating 13:3, 3:1 | | | | | | | 2 | 11 | 44 | 43 | 14 | | | | 114 | 69.9± .39 | 6.2± .28 | 8.9± .40 |

Dwarf plants are considerably more variable than tall plants, as is shown by the coefficient of variability. Crosses 69a and 70a were both grown on the dry farm; these plants show less variability than spring-planted crosses grown under irrigation.

### INTERCROSSING VARIETIES AND THEIR BREEDING BEHAVIOR WITH RESPECT TO DWARFING

In regard to dwarfing in wheat it may be concluded that the 2-factor theory provides a logical explanation for the inheritance of this character. Obviously there are two types of tall varieties: (1) When tall × tall crosses produce dwarfs in $F_2$ and subsequent generations, the genetic composition of the parents is *DDII* and *ddii;* (2) when tall × tall crosses fail to produce dwarfs the parental types are both either *DDII* or *ddii* and, therefore, dwarfs are not to be expected. In addition to these, there appears to be at least one other type, and possibly more. This conclusion is based on a study of intercrossing varieties some of which were known from previous studies to be of a certain genetic composition in respect to the dwarfing character. In Tables 11 and 12 are shown all the crosses upon which the conclusions herein reported are based. In Table 11 are listed those crosses that gave rise to dwarfs in the progeny, and in Table 12 those that did not. Of the 92 crosses, 36 produced dwarfs in the progeny.

TABLE 11.—*Crosses that gave rise to dwarfs in $F_2$ and subsequent generations*

| Cross No. | Female parent | Male parent |
|---|---|---|
| A to G | Dicklow | Sevier. |
| 1 | do | Federation. |
| Q | do | Hard Federation. |
| 23a | Sevier 121 | Dicklow No. 4. |
| 31 | Marquis | Federation. |
| 35 | Dicklow No. 3 | F–22 (Dicklow × Sevier). |
| 36 | do | Sevier No. 59. |
| 39 | Hard Federation | Dicklow No. 3. |
| 40 | Turkey | Federation. |
| 47 | Marquis | Hard Federation. |
| 49 | Hard Federation | Hussar. |
| 50 | Federation | Do. |
| 53 | Martin | IIIc–8 (Dicklow × Sevier). |
| 56 | G–149 (Dicklow × Sevier) | Martin. |
| 57 | Martin | G–149 (Dicklow × Sevier). |
| 67 | Ridit | Sevier No. 59. |
| 68 | do | 15a–318 (Kanred × Sevier). |
| 69 | do | Utac (Dicklow × Sevier). |
| 70 | do | 14–85 (Federation × Sevier). |
| 72 | do | Federation. |
| 76 | Hard Federation | Ridit. |
| 103 | Federation | Martin. |
| No number | Hard Federation | Hohenheimer. |
| 105 | do | Heil Dickkopf. |
| No number | Federation | Do. |
| 107 | do | Hohenheimer. |
| 113 | Ridit | 15a–261 (Kanred × Sevier). |
| 116 | do | 15a–890 (Kanred × Sevier). |
| 117 | do | 16a–1429 (Odessa × Sevier). |
| 118 | do | 16a–1607 (Odessa × Sevier 101). |

TABLE 12.—*Crosses that did not give rise to dwarfs in the progeny*

| Cross No. | Female parent | Male parent |
|---|---|---|
| 4 to 14 | Federation | Sevier. |
| 15 | Kanred | Do. |
| 16 | Odessa | Sevier No. 101. |
| 17 | do | Sevier No. 59. |
| 18a | Federation | Do. |
| 18b | do | Do. |
| 18c | do | Sevier No. 88. |
| 18d | do | Sevier No. 86. |
| 18e | do | Sevier No. 100. |
| 28 | do | F–22 (Dicklow × Sevier). |
| 29 | G–149 (Dicklow × Sevier) | Federation. |
| 30 | Federation | IIIc–18 (Dicklow × Sevier). |
| 32 | Hard Federation | Kota. |
| 33 | Kota | Hard Federation. |
| 34 | Hard Federation | F–22 (Dicklow × Sevier). |
| 38 | G–149 (Dicklow × Sevier) | Hard Federation. |
| 41 | Odessa | Do. |
| 42 | do | Federation. |
| 43 | Hussar | Turkey. |
| 51 | Kanred | Hussar. |
| 54 | Ridit | Kanred. |
| 59 | Hybrid 128 × White Odessa | Do. |
| 66 | do | Sevier No. 59. |
| 71 | Ridit | Turkey No. 989. |
| 73 | Federation | Forward. |
| 84 | do | 5–69–3 (Federation × Sevier). |
| 87 | Hope | Dicklow No. 3. |
| 88 | Preston C. I. 3081 | Federation. |
| 89 | 01–24 [a] | 14–85 (Federation × Sevier). |
| 90 | Dicklow No. 3 | 01–24[a]. |
| 91 | Hope | Federation. |
| 92 | Preston C. I. 3081 | Dicklow No. 3. |
| 93 | do | 01–24[a]. |
| 94 | Marquis | Dicklow No. 3. |
| 100 | Silvercoin | Heil Dickkopf. |
| 101 | Goldcoin | Hohenheimer. |
| 102 | Ridit | Silvercoin. |
| 104 | Goldcoin | Sevier No. 59. |
| 106 | Silvercoin | Do. |
| 108 | do | Kanred. |
| 109 | Hard Federation | Forward. |
| 110 | Forward | Hohenheimer. |
| 111 | do | Heil Dickkopf. |
| 112 | Ridit | 15a–267 (Kanred × Sevier). |
| 114 | do | 15a–400 (Kanred × Sevier). |
| 115 | do | 15a–883 (Kanred × Sevier). |

[a] Probably Dicklow × Federation, parents not known.

## A NEW GENETIC TYPE, *ddII*

In the tall × dwarf crosses it was established that Dicklow and Marquis are of the *ddii* type, while Federation, Hard Federation, and Sevier 59 are of the *DDII* type. Thus, the results of some of the following crosses are difficult to explain if only two types of talls exist:

| | |
|---|---|
| Hope × Dicklow 3 | No dwarfs in progeny. |
| Hope × Federation | Do. |
| Preston × Dicklow 3 | Do. |
| Preston × Federation | Do. |
| 01–24 × Dicklow 3 | Do. |
| 01–24 × Preston | Do. |
| Silvercoin × Heil Dickkopf | Do. |
| Goldcoin × Hohenheimer | Do. |
| Silvercoin × Sevier 59 | Do. |
| Goldcoin × Sevier 59 | Do. |
| Hard Federation × Forward | Do. |
| Hohenheimer × Forward | Do. |
| Federation × Hohenheimer | Dwarfs in progeny. |
| Federation × Heil Dickkopf | Do. |

Since Dicklow 3 is of the *ddii* type, and when it was crossed with Hope no dwarfs appeared, Hope would be expected to be *ddii*. However, the fact that Federation is of the *DDII* type and when crossed with Hope gave no dwarfs proves that Hope is not of the *ddii* type. It is evident that Hope could not be *ddii* in one cross and *DDII* in another. However, if it is considered that Hope is of the new type *ddII*, then dwarfs are not expected in either cross since *ddii* × *ddII* and *DDII* × *ddII* would not throw dwarfs. This is due to the fact that in the first cross there is no dwarfing factor present and in the second cross the inhibiting factor, *II*, would always be present. For the same reason Preston would also be of the *ddII* type.

The fact that Dicklow 3 × 01–24 and 01–24 × 14–85 (Federation × Sevier) gave no dwarfs makes it appear logical to conclude that 01–24 is of the *ddII* type. Federation × Hohenheimer gave dwarfs; therefore, Hohenheimer is of the *ddii* type. However, both Goldcoin (Fortyfold) × Hohenheimer and Goldcoin × Sevier 59 gave no dwarfs; and, furthermore, since Sevier 59 is of the *DDII* type Goldcoin must be of the new type, *ddII*. By a similar analogy Silvercoin and Forward are of the new type, *ddII*. Thus it is apparent that there are at least three genetic types. A genetic classification of strains and varieties into the three types is given in Table 13. The *ddII* type is readily obtained when the other two types, *DDII* and *ddii*, are crossed.

TABLE 13.—*Genetic classification of wheat varieties and strains used in the various crosses, based on their behavior to the dwarfing versus tall characters*

| DDII | ddii | ddII |
|---|---|---|
| Federation | Dicklow | Forward. |
| Hard Federation | Dicklow 3 | Goldcoin. |
| Kota | Dicklow 4 | Hope. |
| Odessa [a] | Heil Dickkopf | Kanred. |
| Sevier | Hohenheimer | Preston 3081. |
| Sevier 59 | Hussar | Silvercoin. |
| Sevier 86 [a] | Marquis | 01–24 (possibly Dicklow × Federation). |
| Sevier 88 [a] | Martin | |
| Sevier 100 [a] | Ridit | 15a–267 (Kanred × Sevier). |
| Sevier 101 [a] | Turkey | 15a–400 (Kanred × Sevier). |
| Utac (Dicklow × Sevier) | Turkey 989 [a] | 15a–883 (Kanred × Sevier). |
| III C–8 (Dicklow × Sevier) | | |
| III C–18 (Dicklow × Sevier) [a] | | |
| F–22 (Dicklow × Sevier) | | |
| G–149 (Dicklow × Sevier) | | |
| 5–69–3 (Federation × Sevier) | | |
| 14–85 (Federation × Sevier) | | |
| 15a–261 (Kanred × Sevier) | | |
| 15a–318 (Kanred × Sevier) | | |
| 15a–890 (Kanred × Sevier) | | |
| 16a–1429 (Odessa × Sevier) | | |
| 16a–1607 (Odessa × Sevier) | | |
| Hybrid 128 × White Odessa [a] | | |

[a] Some of these may be of the new type, *ddII*.

It will be seen from the classification of strains and varieties in Table 13 that strains 15a–267, 15a–400, and 15a–883 are of the new type (*ddII*) coming out of a Kanred (*ddII*) × Sevier (*DDII*) cross, whereas 15a–261 and 15a–890, out of the same cross, are *DDII*.

Strain 01–24 (possibly Dicklow × Federation) is of the new *ddII* type. If Dicklow and Federation are the true parents of 01–24 we have a new genetic type of tall (*ddII*) produced by crossing *ddii* (Dicklow) and *DDII* (Federation).

To determine further whether the proposed genetic composition of the new type is correct, crosses involving dwarfs and the new types were made. Theoretically, the $F_2$ should give a 13:3 ratio and the $F_3$ the usual 7:6:2:1 (7:4:2:2:1) ratio. However, the data from these crosses are not yet available.

It is not to be inferred from the data in Table 13 that this is a final classification. It is possible that some modification may be necessary as additional information becomes available. However, the genetic interpretation proposed provides a logical explanation of the data secured from a detailed study of the 15 crosses as well as from observations on 63 others, involving 50 strains and varieties of wheat.

## SUMMARY

The results of a genetic study of dwarfing in wheat secured from 15 crosses and from observations on the occurrence of dwarfing in 77 others are reported.

Dwarf plants are grasslike clumps and are rather variable, especially as to height, leafiness, and earliness of maturity. Dwarfing is the result of a distinct shortening of either the lower second or third internode or both. Studies in the $F_2$ and $F_3$ generations in four spring-wheat crosses gave results which were explained on a 2-factor basis; that is, a dominant dwarfing factor, *DD*, and an inhibiting factor, *II*.

From a study of the $F_3$ breeding behavior of the tall $F_2$ plants from seven winter-wheat crosses, it was concluded that the dwarfing character could be explained on the usual 2-factor basis.

Results secured from four tall×dwarf crosses gave further proof of the 2-factor theory for dwarfing and also established the genetic composition of a number of varieties in regard to this character.

One tall×dwarf cross gave irregular breeding behavior in $F_3$. The ratio expected was 1 (tall) : 2 segregating : 1 dwarf. It happened that there were too many in the segregating class and too few in the true-breeding dwarf class. These results were explained on the assumption that natural crossing of tall and dwarf plants had occurred in the $F_2$ generation. Data are presented showing that natural crossing of tall and dwarf plants may be of common occurrence.

A biometrical study of the culm length of $F_2$ genotypes, based on the $F_3$ breeding behavior, is presented. The dwarfs in two of the crosses were distinctly outside the range of tall plants. In the other crosses there was considerable overlapping. In length of culm dwarf plants were considerably more variable than tall plants. Tall plants, in spring-wheat crosses grown under irrigation, showed slightly more variation in length of culm than did the tall plants in winter-wheat crosses grown under dry-land conditions.

Intercrossing varieties, some of known genetic composition, gave conclusive evidence that among the tall plants there are more than those of the *DDII* and *ddii* types. The new type is *ddII*. It is readily produced by crossing the other two types (*DDII* and *ddii*). With this additional type it was possible to explain the breeding behavior observed in all the intervarietal crosses studied.

A tentative genetic classification in respect to dwarfing is presented for the varieties and strains used in the crosses.

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